

A biochemical study of the effect of ultraviolet treatment on bovine milk and Cheddar cheese

by

Frans Pieter (Tertius) Cilliers

M.Sc. (Biochemistry)

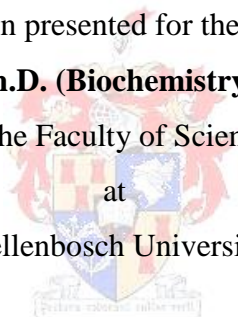
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Promoter: Prof. Pieter Swart

Department of Biochemistry, Stellenbosch University

Co-promoter: Prof. Pieter Gouws

Department of Food Science, Stellenbosch University

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Summary

This study describes:

1. The evaluation of a novel, patented thin-film, turbulent-flow Ultraviolet disinfection system as an alternative processing method to thermal pasteurisation for the disinfection of bovine milk.
2. The microbial, biochemical and sensory characterization of bovine milk treated by heat and Ultraviolet light and then used for the commercial production of Cheddar cheese.
3. The microbial, biochemical and sensory characterization of commercial Cheddar cheese produced from bovine milk treated by heat and Ultraviolet light.

Opsomming

Hierdie studie beskryf:

1. Die evaluasie van 'n unieke, gepatenteerde dun-film, turbulente vloei Ultravioletsisteem as 'n alternatief vir termiese pasteurisasie vir die behandeling van beesmelk.
2. Die mikrobiologiese-, biochemiese- en sensoriese karakterisasie van beesmelk behandel met hitte en Ultravioletlig gebruik vir kommersiële produksie van Cheddar kaas.
3. Die mikrobiologiese-, biochemiese- en sensoriese karakterisasie van kommersiële Cheddarkaas vervaardig van beesmelk wat behandel is met hitte en Ultravioletlig.

Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and has not previously in its entirety or in part been subjected to any university for a degree.

1st September 2015

F.P. Cilliers

Date

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Outputs of the Ph.D. study

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List of Abbreviations and Acronyms

ADV	Acid Degree Value
AMS	Aerobic Mesophilic Spores
ANMS	Anaerobic Mesophilic Spores
ANOVA	Analyses of Variance
ANTS	Anaerobic Thermophilic Spores
APC	Aerobic Plate Count
ATS	Aerobic Thermophilic Spores
CIP	Cleaning in Place
COP	Cholesterol Oxidation Products
DMS	Dimethyl Disulfide
ESL	Extended Shelf Life
EV	Error Values
FA	Fatty Acids
FAD	Flavin adenine dinucleotide
FFA	Free Fatty Acids
FMN	Flavin mononucleotide
GC	Gas Chromatography
GCMS	Gas Chromatography Mass Spectrometry
HTST	High Temperature Short Time
LCMS	Liquid Chromatography Mass Spectrometry
LSD	t-Least Significant Difference
LTLT	Low Temperature Long Time
MA	Malonaldialdehyde
MAP	Milk Alkaline Protease
MP	Median Polishing
P	Pasteurised Milk
P ₀ , P ₆₀ , P ₆ , P ₁₂	Cheese manufactured from Pasteurised Milk only at day 0, day 60, after 6 months and after 12 months respectively
PG	Plasminogen
PHE	Plate Heat Exchanger

PL	Plasmin
PUFA	Poly Unsaturated Fatty Acids
RM	Raw Milk
RV	Residual Value
SIM	Single Ion Monitoring
SP-4	SurePure 4 Turbulator™ System
SP-40	SurePure 40 Turbulator™ System
SPME	Solid Phase Micro Extraction
STDEV	Standard Deviation
TBARS	Thiobarbituric Acid Reactive Substances
TCA	Trichloroacetic Acid
TMS	Trimethylsilyl
TNBS	Trinitrobenzene Sulfonic Acid
UHT	Ultra High Temperature
UV	Ultraviolet treated Milk
UV ₀ , UV ₆₀ , UV ₆ , UV ₁₂	Cheese manufactured from Ultraviolet treated milk only at day 0, day 60, after 6 months and after 12 months respectively
UV	Ultraviolet treatment of Milk followed by Pasteurisation
UVP ₀ , UVP ₆₀ , UVP ₆ , UVP ₁₂	Cheese manufactured from Ultraviolet treated milk followed by Pasteurisation at day 0, day 60, after 6 months and after 12 months respectively
UVT	Ultraviolet Transmittance

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In loving memory of my Mom, Marianne Cilliers

“Dans les champs de l'observation le hasard ne favorise que les esprits préparés.”

(In the fields of observation chance favours only the prepared mind.)

Pasteur, University of Lille, 7th December 1854

Chapter 1

Introduction

April 20th, 1862 can be regarded as a milestone in scientific discovery, when the father of the ‘germ theory’, the French scientist Louis Pasteur and his research assistant Claude Bernard, first successfully applied heat to liquids to kill microorganisms. At the time it was hypothesized that the ‘invisible enemy’ or microorganisms were the main cause of spoilage of liquids such as beer, wine and milk. The first tests conducted were on request of Napoleon III, whom asked Pasteur to apply his knowledge in order to try and extend and preserve the quality of French wine. The research methodology followed and process developed would later be successfully applied to other liquids such as vinegar and milk, and still has practical application within the food industry today. The process of preserving liquids through applied heat or partial sterilization would later be named after Pasteur, whom throughout his career took a pragmatic view and an active interest in the development of industrial applications. He confirmed this viewpoint by stating: *“There are no such things as pure and applied science. There are only science and the application of science.”* (Debré, 2000).

A few decades after Pasteur’s discovery, an Icelandic physician by the name of Niels Ryberg Finsen started using ultraviolet light for the external treatment of diseases associated with the skin and the mucus membranes. Finsen suffered from a condition known as Pick’s disease, a disease characterized by the progressive thickening of connective tissue of membranes, resulting in impairment of some organ functions. This condition, he later admitted, was one of the main reasons why he started experimenting with light, especially sunlight, as he was curious as to the possible benefits it could have. The Nobel Prize in Physiology or Medicine in 1903 was awarded to Niels Ryberg Finsen *“...in recognition of his contribution to the treatment of diseases, especially lupus vulgaris, with concentrated light radiation, whereby he has opened a new avenue for medical science”* (Anonymous, 2014). Upon being awarded the Nobel prize for his exemplary work in this field in 1903, he proclaimed

“Well, thus it has now been established that the thing is Danish...” which was unfortunate as he could not attend the prize giving function in Stockholm that year due to his illness. Some of his most important work include the publications “*Om Lysets Indvirkninger paa Huden* (On the effects of light on the skin)” in 1893 and “*Om Anvendelse i Medicinen af koncentrerede kemiske Lysstraaler* (The use of concentrated chemical light rays in medicine)” in 1896. By the mid 1930’s based on the work of Finsen, the use of UV light in medicine was well established to treat conditions such as mumps and other skin infections (Douglas and Campbell, 1996).

Milk and milk constituents have always provided a model system for scientific investigation for various reasons. Milk is a nutrient rich product, which is secreted by the female of all mammalian species, primarily to meet the nutritional requirements of the neonate. Milk contains lipids (which include essential fatty acids), proteins and peptides, essential amino acids, immunoglobulins, enzymes, enzyme inhibitors, growth factors, hormones and anti-bacterial agents, lactose, vitamins and inorganic elements such as calcium and water (Fox & McSweeney, 1998). Since prehistoric times humans have used milk in various ways: to drink, to produce cheese and other fermented milk products (such as buttermilk and yoghurt), to churn into butter or for use in combination with other food ingredients for the manufacturing of baked goods, ice cream and frozen yoghurts and confectionary products. As a result the safety and quality of milk, either as an ingredient or for direct consumption, is of paramount importance (Frank, 1997; Fox & McSweeney, 1998; Jay, 2000; Jay *et al.*, 2005).

In the dairy industry, pasteurisation plays an important role in the preservation of primary and secondary milk products, by destroying pathogenic bacteria and other spoilage organisms. Illnesses from contaminated milk and milk products have occurred worldwide ever since cows have been milked. In the 1900’s it was discovered that milk can transmit tuberculosis, brucellosis, diphtheria, scarlet fever, and Q-fever to humans (Burton, 1986; Bramley & McKinnon, 1990; ICMSF, 1998; Spreer, 1998; Jay *et al.*, 2005). Fortunately, the threat of these diseases and the incidence of outbreaks involving milk and milk products have been greatly reduced due to improved sanitary milk production practices and pasteurisation. The main purpose of pasteurisation is the destruction of food-borne pathogens such as *Brucella abortis*, *Mycobacterium tuberculosis*, *Mycobacterium avium* spp. *paratuberculosis*

and *Coxiella burnetii* and as a secondary purpose pasteurisation destructs spoilage microorganisms and enzymes (Enright *et al.*, 1957; ICMSF, 1998; Ranjith, 2000; Jay *et al.*, 2005).

As a result of the above mentioned, food safety is of significant importance to the dairy sector. At present there is a growing interest in alternative sterilization technologies, such as UV radiation, for food preservation, specifically for the treatment of liquids, such as milk and whey (Koutchma, 2009). The wavelength of 254 nm (UV-C) has the highest germicidal effect, and damages the DNA of exposed cells by causing thymine dimer formation in the microorganisms' DNA molecules, thereby destroying the microorganisms' ability to grow. This technology presents numerous benefits over traditional sterilization systems, such as heat treatment (pasteurisation), as it for example does not produce undesirable by-products, has a low cost of installation, no chemical input is needed, is low in maintenance and production cost and emitting less carbon emissions in relation to traditional thermal pasteurisation systems (Butz & Tauscher, 2002; Jay *et al.*, 2005; Koutchma *et al.*, 2009).

The study focused on new generation, turbulent-flow UV treatment systems that have been designed to optimize exposure to microorganism target populations. Such systems transfer UV-C energy directly, selectively and efficiently to the microbial DNA in order to limit secondary effects on the biochemical composition and the nutritional value of the liquid being treated (Knorr, 1999; Koutchma, 2009; Koutchma *et al.*, 2009; Simmons *et al.*, 2012). The application of UV treatment is both feasible and challenging. Excessive UV exposure can lead to oxidation and sensory defects in liquids, therefore optimization of critical design elements in UV treatment systems, are very important (Koutchma *et al.*, 2009; Rossito *et al.*, 2012). UV-C irradiation has shown to be capable of reducing microorganisms in the liquids being treated, however limited research has been done to quantify the effects of such a treatment regime (Guerrero-Beltrán & Barbosa-Cánovas, 2004; Koutchma, 2009). Further elucidation of the effect of UV-C treatment on the biochemical character of the liquids being treated and/or products being manufactured from UV treated liquids, such as during cheese manufactured from UV treated milk, remains important.

The hypothesis is that non-thermal alternative processing technologies, such as UV treatment, could present numerous advantages within the dairy sector with the treatment of milk in comparison to traditional thermal pasteurisation. For example the microbial inactivation results achieved with the UV-treatment of raw milk prior to cheese manufacture, opens various opportunities for such treatment in the cheese manufacturing process, either on its own or in conjunction with pasteurisation. The hypothesis is that with UV treatment alone, the character of the cheese manufactured, will more likely resemble the character of cheese produced from raw milk. In addition UV treatment could possibly also attribute to limit defects occurring in the cheese, associated with high initial bacterial counts in raw milk, such as: Higher yield due to the reduction of bacteria, such as psychrotrophs, in the milk from farms; the possible reduction of proteinases and lipases from bacterial origin; the reduction of flavour defects (fruity, stale, bitter, putrid, rancid); the reduction of final product non-conformances; shorter ripening period, with controlled maturation and limitation of off-flavour development over time and enhanced safety of the final product when used as an adjunct to pasteurisation. Furthermore alternative processing technologies may have fewer organoleptic consequences when compared to thermal pasteurisation and due to the energy efficiency and low investment cost could allow a sustainable processing solution for smaller and subsistence farmers. The dissertation focuses on the application of UV-C radiation within the dairy industry using a novel thin-film, turbulent flow UV treatment system.

Chapter 2 delivers an overview of the principles of UV technology, with specific emphasis to the application of UV treatment of milk and the use of UV irradiated milk for the manufacturing of Cheddar cheese. The chapter also defines UV light, the mechanism of action and highlights the critical control parameters to keep in mind when applying the technology to liquid products for the purpose of pasteurisation or sterilization (Guerrero-Beltrán & Barbosa-Cánovas, 2004; Koutchma, 2009). A brief overview is presented on current dairy products processing regimes, focusing on thermal pasteurisation, and comparing it to UV radiation as an alternative processing technology. Furthermore milk composition with special mention to the role of macro- and micronutrients and micro flora present in raw milk are briefly discussed, as these components forms the basis of quantitative and qualitative studies involving the investigation into alternative processing technologies.

Chapter 3 describes the development of the SurePure thin-film, turbulent flow UV treatment system, which has been used in this research study. The review focuses on the system's product design characteristics, and the hydrodynamic model for the treatment of turbulent liquids, which differentiates the SurePure system from other conventional UV processing systems (Simmons *et al.*, 2012). The research conducted lead to the successful registration of a patent on the SurePure TurbulatorTM system, which is listed in Annexures A and B.

Chapter 4 describes the inactivation kinetics of microorganisms, including the germicidal effect of UV-C irradiation on relevant and important pathogens in liquid raw milk using the SurePure TurbulatorTM system. The content of the chapter was presented in a written presentation in 2006 at the American Society of Agricultural and Biological Engineers (Portland, Oregon, United States) describing new methods for UV treatment of milk for improved food safety and product quality.

Chapter 5 describes the research conducted and submitted as a peer review publication in *Innovative Food Science and Emerging Technologies* describing a biochemical study to quantify the changes in the liquid dairy products being treated by UV-C irradiation as well as comparing it to the biochemical character of liquid dairy products being treated with conventional processing systems, such as thermal pasteurisation.

Chapter 6 compares the microbial and biochemical character of commercially produced Cheddar cheese during maturation, as a result of different treatments applied to the milk before cheese manufacture. The treatments included UV-C treatment, heat pasteurisation and a combination of the UV-C treatment and heat pasteurisation. The results of the study were submitted for peer review in *Innovative Food Science and Emerging Technologies*.

Chapter 7 summarizes the main conclusions of the research and also looks at possible future development opportunities using UV radiation as an alternative processing technology to current standard and accepted processing protocols followed in the dairy and related food industries.

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Chapter 2

Literature Review

2.1. Introduction

Since the beginning of 21st century, there has been an increase into the investigation of “*novel food processing*” technologies, specifically aimed at replacing and/or extending traditional practices followed such as heating, chemical preservation, refrigeration, drying, salting and smoking. Alternative non-thermal processing methods include high pressure processing (HPP), pulsed electric fields (PEF), ultrasound, no-ionizing radiation such as UV, ionizing radiation such as electron and gamma rays treatment or a combination of the above mentioned (Kessler, 2002; Guerrero-Beltrán & Barbosa-Cánovas, 2004; Koutchma 2009; Koutchma *et al.*, 2009).

Recent advances in the science and engineering of UV light radiation have demonstrated that this technology holds considerable promise as an alternative to traditional thermal pasteurisation for liquid foods and food ingredients. Although the application of UV for the treatment of liquids is still limited due to the low UV transmittance in turbid liquids, advances in reactor and lamp design features are expanding possibilities for the use of UV as a disinfectant in the food industry (Guerrero-Beltrán & Barbosa-Cánovas, 2004; Koutchma 2009; Koutchma *et al.*, 2009). The primary advantages associated with UV treatment of liquids include the extension of product shelf life and increased quality and safety of the final products being produced. In addition, as UV is seen as a less invasive technology, it could negate some of the negative effects associated with traditional thermal processing systems and other preservations methods (Bintsis *et al.*, 2000; Koutchma, 2009).

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) of the USDA in 2004 altered the definition of “pasteurisation” to include any process, treatment, or combination thereof, which is applied to food to reduce microorganism(s) of public health significance. The processes and technologies described in the NACMCF report included UV irradiation as an alternative to heat that can be used for pasteurisation purposes (Koutchma, 2009).

Within the food and related industries, the application of UV radiation can be divided in 3 broad categories (Bintsis *et al.*, 2000):

- The inhibition of microorganisms on surfaces
- The destruction of microorganisms in the air and
- The sterilization of liquids

The application of UV radiation is well described for the disinfection of surfaces and air, however, research are more limited when evaluating liquids other than water. The study therefore focuses exclusively on novel applications of UV radiation within liquids, specifically liquid milk as the key ingredient to all value added dairy products. In regards to the use of UV radiation of liquids, literature reviews of Guerrero-Beltrán & Barbosa-Cánovas (2004) and Koutchma (2009) are valuable sources of information. In Table 2.1, the possible main advantages of UV processing of liquid products as recorded by various authors are summarized.

In addition to the benefits specified in Table 2.1, further potential application benefits identified using UV radiation in the dairy industry as an alternative processing technology pasteurisation, include the following (Koutchma *et al.*, 2004; Matak *et al.*, 2005; Reinemann, *et al.*, 2006; Koutchma *et al.*, 2007; Keyser *et al.*, 2008; Donaghy *et al.*, 2009):

- Cold treatment of raw milk,
- Reduction of bacteria not susceptible to thermal treatment,
- Reduction of psychrotrophic bacteria in refrigerated milk stored for prolonged periods,

- Bacteria reduction to improve milk quality in developing countries where lack of a reliable energy supply and high cost makes on-farm refrigeration prohibitive.

Table 2.1: Main advantages of processing liquid food products with UV technology.

Main advantage of UV processing identified	Source
Increased shelf-life of the product	Koutchma, 2009
Reduction of carbon footprint	Koutchma, 2009
Reduction of waste	Koutchma, 2009
Reduced energy consumption (Cold process)	Koutchma, 2009; Bachmann, 1975; Morgan, 1989
Reduced water consumption	Koutchma, 2009
Increased organoleptic quality	Koutchma, 2009
Positive consumer image	Koutchma, 2009
No undesirable by products during processing	Chang <i>et al.</i> , 1985; Guerrero-Beltrán & Barbosa-Cánovas, 2004
No residual radioactivity as ionising irradiation	Guerrero-Beltrán & Barbosa-Cánovas, 2004
Low CAPEX and installation cost in comparison with other sterilisation methods	Koutchma, 2009; Bachmann, 1975
No chemical input needed	Guerrero-Beltrán & Barbosa-Cánovas, 2004
Low maintenance and production cost	Guerrero-Beltrán & Barbosa-Cánovas, 2004
No chemical residues produced	Bachmann, 1975; Guerrero-Beltrán & Barbosa-Cánovas, 2004

2.2. Ultraviolet Definition and Classification

Ultraviolet (UV) light is short-wave, electromagnetic light in the electromagnetic radiation spectrum, with typical wavelengths of between 100 – 400 nm. On either side of the visible wavelengths in the spectrum, are the invisible long waves such as radio waves and invisible short waves such as UV rays, X-rays and cosmic rays. UV light can be divided into 4 definitive subclasses, namely the vacuum UV range (100 – 200 nm), UV-A (315 – 400 nm), UV-B (280 – 315 nm) and UV-C (200 – 280 nm) as indicated in Figure 2.1 (Snowball & Hornsey, 1988; Bintsis *et al.*, 2000; Sastry *et al.*,

2000). The latter is called the germicidal UV range, as it will inactivate bacteria, viruses and protozoan microorganisms, if such organisms are to be exposed to UV-C radiation. UV light in general is regarded as a non-ionizing radiation technology, as it demonstrates low penetration power due to inherent low energy of photons compared with ionizing irradiation (Kyzlink, 1990).

UV radiation is the most readily available source of radiation that is produced by arc discharges in mercury tubes. Radiative transfer is defined as the process during which light or other electromagnetic energy is transmitted from one form to another (i.e. through absorption, scattering, reflection etc.). The absorption of non-ionizing radiation induces excitation of atoms and/or molecules (Kyzlink, 1990). Light is emitted from the gas discharge from UV lamps at wavelengths dependent upon its elemental composition and the excitation, ionization, and kinetic energy of those elements. Other types of radiation that can be used include X-ray, gamma- and beta radiation¹ (Snowball & Hornsey, 1988; Kyzlink, 1990; Sastry *et al.*, 2000; Bintsis *et al.*, 2000).

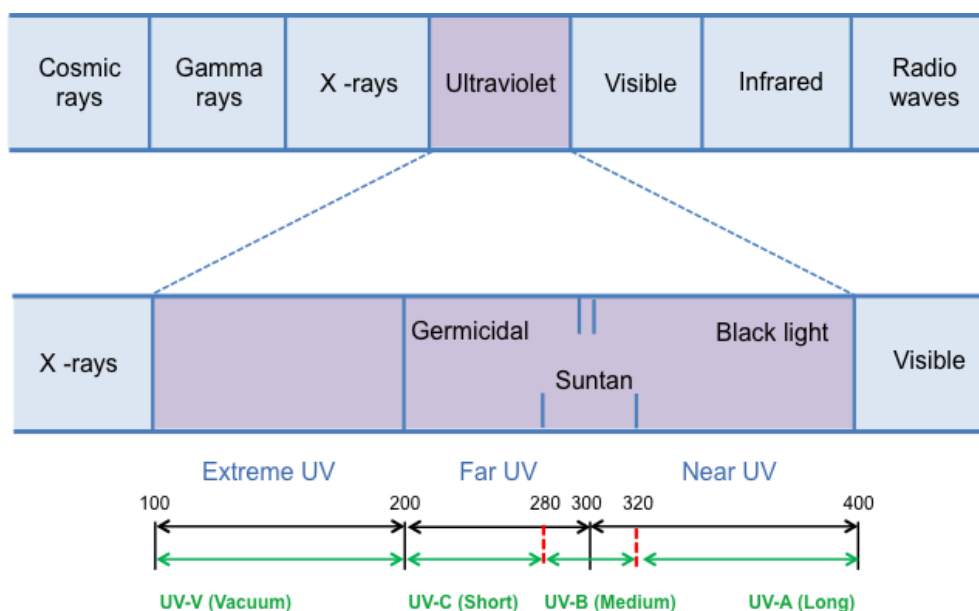


Figure 2.1: Electromagnetic spectrum (adapted from Snowball & Hornsey, 1988).

A comprehensive review of the mechanisms of UV light generation, basic principles of photochemistry and UV radiation energy is given by Koutchma *et al.* (2009), which was not included in this dissertation, but will serve as good reference on the fundamental principles of UV light propagation. However, it is important to elucidate the differences between the adsorption, reflection, scattering and refraction as mechanism of UV light propagation within the medium being treated. The difference in terms of these definitions and therefore the resulting effect on energy absorption as a result of UV light exposure is diagrammatically explained in Figure 2.2.

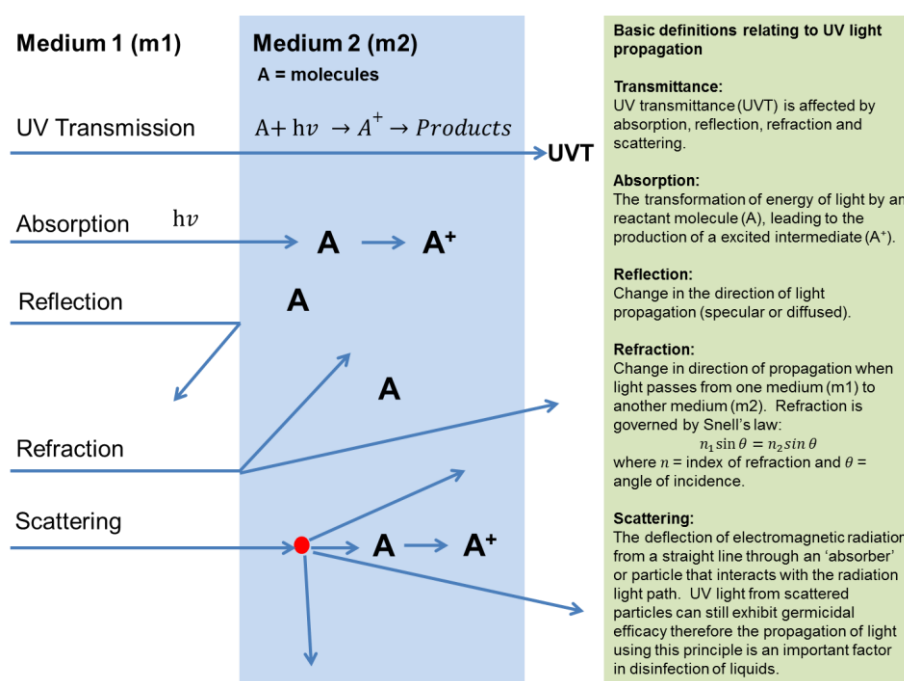


Figure 2.2: Diagrammatic representation to illustrate the differences between the absorption, reflection, refraction and scattering of UV light (Koutchma *et al.*, 2009).

2.3. Mechanism of microbial inactivation by ultraviolet light

UV radiation absorbed by the microorganisms' DNA will impair cell proliferation and growth and will ultimately lead to cell death (Liltved & Landfald, 2000; Guerrero-Beltrán & Barbosa-Cánovas, 2004). UV with a wavelength of 228 – 265 nm is the most deadly to microorganisms because this is the ideal absorption spectrum of the organisms' nucleic acids (Bachmann, 1975; Shama, 1999). The germicidal efficacy at these wavelengths will be effective against microorganisms such as bacteria, viruses,

protozoa, moulds, yeasts and algae (Morgan, 1989; Sizer & Balasubramaniam, 1999; Bintsis *et al.*, 2000). At these wavelengths, the nucleic acids of the organism exposed to UV-C will absorb the radiation, which causes lethal mutations in the DNA. At wavelengths above 300 nm the germicidal effect is limited and/or completely negated (Bachmann, 1975).

2.3.1. Dimerization of cellular DNA

The germicidal effect of UV radiation is mainly as a result of the dimerization of DNA of the microorganism being exposed to the UV radiation (Moan & Peak, 1989). As indicated in Figures 2.3 and 2.4, the changes in the DNA structure are as a result of cross-linking between thymine and/or cytosine (pyrimidine) nucleoside bases in the same DNA strand forming thymine dimers, which is proportional to the UV-C dose applied (Kyzlink, 1990; Ray & Bhunia 2014). The formation of thymine dimers in the DNA impairs DNA transcription and replication, therefore inactivating the microorganisms' cellular and physiological functions. Structural damage of cells exposed to UV, includes the rupturing of cell membranes, mechanical damage to cell walls and breaking of DNA strands (Lado & Yousef, 2002). Following UV radiation, lethal DNA lesions are scattered throughout the population of cells. The cells that are not able to repair the UV-induced damage to their DNA, and will not be able to further replicate or transcribe. Cells that were damaged, but still survive, usually undergo mutations (Snowball & Hornsey, 1988; Sastry *et al.*, 2000; Lado & Yousef, 2002). UV radiation can also cause a small amount of protein to denature. This happens when aromatic amino acids are cross-linked at their carbon-carbon double bonds. The denaturing of the proteins causes the cell membrane to be depolarized and it also changes the ionic flow in the cell wall (Lado & Yousef, 2002). Other photoproducts formed include pyrimidine adducts, spore photoproducts DNA-protein cross-links, pyrimidine hydrates and strand breakage interstrand crosslinks (Harm, 1980).

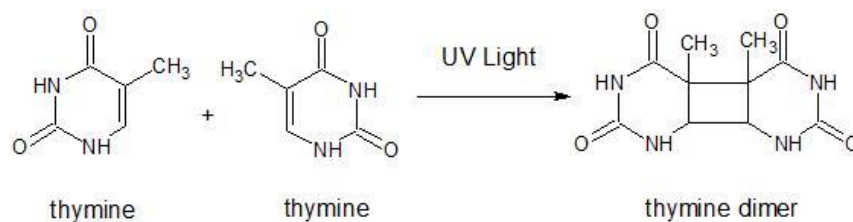


Figure 2.3: Cross-linking between thymine (pyrimidine nucleoside bases) to form a thymine dimer.

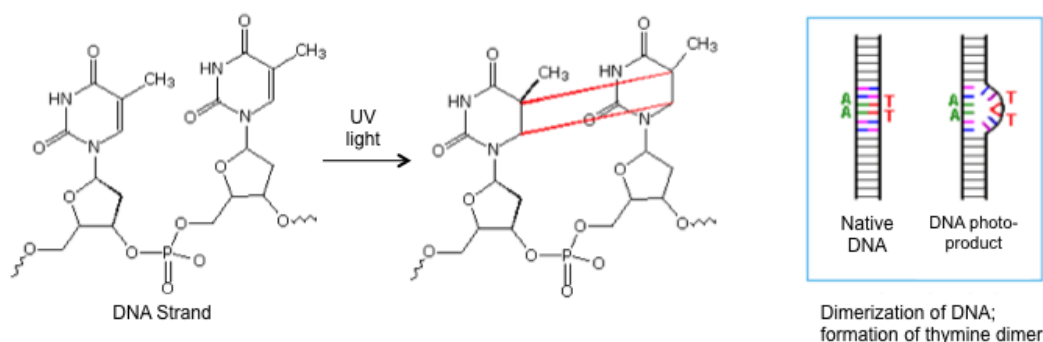


Figure 2.4: Dimerization of DNA following exposure to UV-light.

In contrast to Bachman's (1975) observations, UV radiation with longer-wavelength UV-A (320-400 nm) can cause the formation of hydroperoxide radicals in the membrane's fatty acids. These radicals can change the permeability of the membrane (Lado & Yousef, 2002). When key enzymes are inactivated or membrane selectivity is disabled, the cell loses its physical properties and cannot function correctly (Lado & Yousef, 2002).

2.3.2. Photoreactivation

Microorganisms possess several recovery mechanisms to limit the effect of UV radiation and to promote cell survival. These processes present a potential hazard, as sub-lethal stresses can cause the expression of cell-repair systems. The exposure of a cell to UV can induce enzymatic photo repair, as well as the expression of excision-repair genes that remove damaged nucleic acids and replace them with the functional nucleic acids. For example, *Escherichia coli* that can survive UV radiation usually

has a gene called *htpR*, which acts as a transcriptional regulator that is used to repair stress damage to the cell following UV radiation (Snowball and Hornsey, 1988; Sastry *et al.*, 2000; Lado & Yousef, 2002). Cells that are adapted to stress-conditions are a particular hazard to the food and beverage industry, since these organisms may survive UV processes used as a stand-alone technology or in combination with other preservation technologies (hurdle technology). A further hazard is that the repeated exposure of cells at sub-lethal UV dosages can cause the formation of particularly resistant mutants, which will be very difficult to eradicate using the preservation method of choice (Lado & Yousef, 2002). Table 2.2 compares the effective UV dose needed (J.m^{-2}) to achieve a 4-log reduction in the target organism treated with and without photoreactivation (Hoyer, 1998).

Table 2.2: UV 254 nm radiant exposure (J.m^{-2}) for a 4-log reduction of microorganisms, comparing exposure required with photoreactivation (adapted from (Hoyer, 1998)).

Microorganism	Exposure required without photoreactivation (J.m^{-2})	Exposure required with photoreactivation (J.m^{-2})
<i>Escherichia coli</i> ATCC 11229	100	280
<i>E. coli</i> ATCC 23958	50	200
<i>E. coli</i> NCTC 5934	90	215
<i>E. coli</i> NCIB 9481	100	180
<i>E. coli</i> wild isolate	110	270
<i>Enterobacter cloacae</i>	100	330
<i>Klebsiella pneumoniae</i>	110	310
<i>Citrobacter freundii</i>	80	250
<i>Yersinia enterocolitica</i>	100	320
<i>Salmonella</i> Typhi	140	190
<i>Salmonella</i> Typhimurium	130	250
<i>Serratia marcescens</i>	130	300
<i>Enterocolitica faecium</i>	170	200
<i>Vibrio cholerae</i> wild isolate	50	210
<i>Pseudomonas aeruginosa</i>	110	190
<i>Mycobacterium smegmatis</i>	200	270
Polio virus (Mahoney)	290	-
Rotavirus SA 11	350	-
<i>Staphylococcus aureus</i> phage A994	380	-

Photoreactivation through exposure of UV-C injured organisms to wavelengths greater than 300 nm, occurs when the organism being exposed are capable of repairing the structural DNA damage through the expression of the single-strand DNA binding protein, RecA, and the activation of cyclobutane pyrimidine (CPD) photolyase, an enzyme that monomerises the DNA cyclobutane dimers formed after UV radiation (Stevens *et al.*, 1998; Sinha & Häder, 2002). CPD consist of two coenzymes, namely flavin adenine dinucleotide (FAD) factor and either methenyltetrahydrofolate (MTHF) or 8-hydroxy-5-deazariboflavin types (Carell *et al.*, 2001). Following exposure to visible light (in the near UV blue spectral range between 310 – 480 nm), CPD photolyase repair the lesion caused in the DNA by UV radiation, and should therefore be avoided by using dark packaging and refrid if geration of the product after UV treatment if possible. The MTHF or 8-hydroxy-5-deazariboflavin absorbs the electromagnetic energy through the absorption of visible light and then transfers the energy to the FAD factor which in turn is reduced and transfers the electron to the dimer which is then divided and repaired. Oguma *et al.* (2002) used endonuclease sensitive site (ESS) assays to determine the number of UV induced pyrimidine dimers formed after UV exposure on *E.coli*. They concluded that UV-C (wavelength between 220 – 300nm) reduced the photo repair of ESS, possibly due to inactivation of the endogenous photolyase (*in vivo*). Süß *et al.* (2009) developed a rapid PCR-based analytical method with success to investigate photo repair mechanisms in microorganisms following UV radiation.

2.3.3. Dark repair

In contrast with photoreactivation, dark repair in the microorganism does not need exposure to visible light in order to be activated. Under stress conditions, such damage caused to the DNA of the cell following exposure to UV light, the RecA protein in the cell is activated. The activated RecA protein then cleaves the negative regulator, LexA protein, which represses the transcription of the genes involved in the SOS signal in the cell. As LexA are lowered, the SOS system in the cell is activated, which in turn is responsible for the dark repair mechanism (Aksenov, 1999). The SOS system is responsible for the activation of various genes involved in dark repair mechanisms and includes the excision base pair repair, nucleotide excision repair,

recombinant repair, mutagenic repair, phage control repair and multiplicity reactivation in viruses. As the repair mechanisms are activated the level of the activated RecA protein is lowered, and the concentration of the LexA protein are again increased to normal levels associated within the cell. The induction of the LexA gene in organisms are very specific and can be attenuated at UV-C dosage levels of between 100 – 600 J.m² (Jungfer *et al.*, 2008).

2.3.4. Inactivation kinetics

Whether using heat or light to inactivate bacteria, the changes in bacterial levels will follow first-order kinetics. Inactivation of bacteria with heat in pasteurisation is based upon the common principle in food safety of a D-value (Jay, 1986; Koutchma, 2009; Rossito *et al.*, 2012).

First-order kinetics state that the rate of change in the concentration of an agent (microorganisms in this instance) is dependent only on the concentration of that agent with all other components of the system being kept constant (for heat this would be holding time and temperature constant so that there will be a predictable killing effect each time heat is applied). The definition of a D-value is the time it takes to create a 1-log or 90% reduction in the level or concentration of a microorganism. D-values will be different for different bacterial species and can also change as the temperature and holding time changes (Koutchma, 2009; Koutchma *et al.*, 2009; Rossito *et al.*, 2012). If all conditions are kept constant, the D-value can be used to predict levels of microorganism inactivation and have assurances for margins of safety when applying a given UV-C dose. The linear model of bacterial inactivation is based on first order kinetics. The mathematical representation of this is:

Equation 1
$$r = - \frac{d[A]}{d[P_{uv}]} = k[A]$$

where r is the rate, k is the first order rate constant with the units of 1/ P_{uv} (UV-C dosage) is measured. A is the concentration of microorganisms and d represents the change or $d[A]$ the change in concentration of A (microorganisms) in this study.

The integrated first-order rate law can then be calculated as follow:

Equation 2
$$\log[A] = -k[Puv] + \log[A]_0$$

Since k is the slope of the line in first order kinetics, to solve the equation for D-value, the equation is rearranged to:

Equation 3

$$\frac{P_{uv}}{D} = \frac{\log [A]}{\log [A]_0}$$

From this the D-value can be calculated by taking the reciprocal of the slope of the line from the logarithmic killing plots.

The UV-C dose required (measured at a wavelength of 253.7 nm) to cause an order of magnitude reduction in the microorganism population (D-value), ranges from a few mJ.cm^{-2} for most bacteria to several hundred for algae and fungi (Kouchma, 2009).

However, as reported by Guerrero-Beltrán and Barbosa-Cánovas (2004) and Rossitto *et al.* (2012), the kill-rate and survival of some microorganisms following UV-C radiation, can also be presented as a sigmoidal function. This is applicable in instances where the ‘plateau regions’ of the sigmoidal curve is extended, and as such more complex mathematical models should be used to calculate the UV-C dose response. The sigmoidal curve will consist of an initial plateau region, indicating injury to the microbial population, a linear portion, illustrating kill-rate as per the first-order reaction kinetics and a final plateau (or tailing), indicating survival of organisms following UV exposure (as indicated in Figure 2.5) (Anonymous, 2012). The latter is also dependent on the type of liquid being treated and according to Guerrero-Beltrán & Barbosa-Cánovas (2004), is most probably related to the percentage soluble and insoluble solids of the liquid being treated. However other factors should also be considered for this phenomenon, such as the type of microorganism irradiated, the length and intensity of UV radiation and the number of initial organisms present.

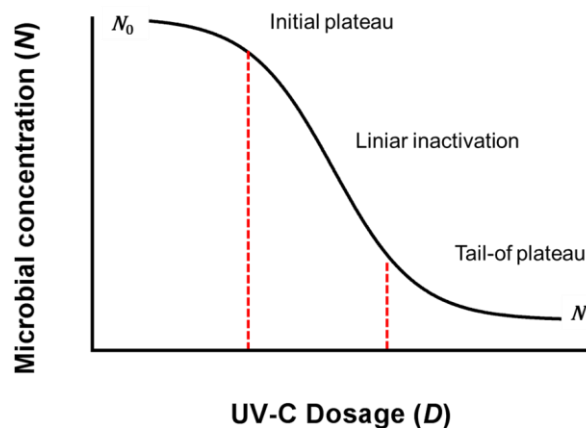


Figure 2.5: Survival-curve (sigmoidal) of microorganisms following UV-C exposure, where N_0 indicates the initial number of microorganisms and N is the final number of microorganisms.

2.3.5. UV-C irradiance and UV-C dose

As the light passes from the UV-C lamp through to the liquid, all the materials it passes through according to the Lambert-Beer law attenuate it. The irradiance (or fluence rate, I) falls exponentially with path length (x) from its initial value (I_0) for a given material with absorption coefficient (α) according to the following equation (Guerrero-Beltrán & Barbosa-Cánovas, 2004; Koutchma 2009; Koutchma *et al.*, 2009):

Equation 4

$$\frac{I}{I_0} = e^{-\alpha x}$$

The absorption coefficient varies according to the type of liquid being treated. The absorption coefficient for full-cream milk will be approximately 290 to 300 cm^{-1} (in comparison to water at $\sim 0.01 \text{ cm}^{-1}$) as reported by Kouchma, (2009) and Simmons *et al.* (2012).

The exposure of organic molecules to UV-C light will induce chemical changes within the organic compounds. The extent of the change, which will determine the germicidal efficacy, depends on various factors such as the quantum yield (the

number of organisms destroyed divided by the number of photons absorbed by the system and the energy yield of the incident photons) and the structure of the molecules in question. The UV-C dosage, also referred to as the UV Irradiance or UV intensity flux, is defined as (Bintsis *et al.*, 2000; Guerrero-Beltrán & Barbosa-Cánovas, 2004):

Equation 5
$$D = I_{254} \times t$$

where D is the effective UV-C Dosage (J.m^{-2}), I_{254} is the intensity (W.m^{-2}) and t is the exposure time (s) (Chang *et al.*, 1985; Morgan, 1989; Stevens *et al.*, 1999). The UV-C dosage can be confirmed through various methods, such as chemical actinometry, which is well described in the research of Altic *et al.* (2007) and irradiance biodosimetry, which consist of measuring the dose response under specific conditions in fluid inoculated with surrogate microorganism (Sastry *et al.*, 2000). UV irradiance may be monitored by radiometers, either thermal or photonic, UV sensors, or actinometer; however, the most reliable method is still biodosimetry.

2.4. Factors influencing ultraviolet inactivation of microorganisms

The inactivation of microorganisms following UV exposure will depend mainly on three factors according to Altic *et al.* (2007). The resistance of the microorganism to UV radiation and the type of microorganism treated, secondly the absorption properties of the medium treated in which the microorganisms are suspended and thirdly the UV dose applied to the medium being treated (Altic *et al.*, 2007). In addition to Altic *et al.*'s (2007) three factors the process characteristics including the source of UV light, the flow dynamics and flow velocity and resulting exposure time (residence time) is important as well. Sastry *et al.* (2000) reported that all parts of the fluid (or liquid being treated) should be exposed to at least 400 J.m^{-2} of UV-C at 254 nm to reduce initial population to 5-log (as measured by surrogate microorganisms) in order to achieve microbiologically safe end product (Sastry *et al.*, 2000; Guerrero-Beltrán & Barbosa-Cánovas, 2004). Furthermore Sastry *et al.* (2000) also highlighted two further important points, namely that the UV Dose need to be applied to the entire food system, thus the process design and treatment regime, need to ensure that the liquid is treated homogenously. Second, that the exposure time needs to be adjusted

to achieve proper germicidal efficacy on the target microorganism or population being treated.

2.4.1. Type of microorganism

According to Kyzlink (1990) in order for UV radiation to have the desired germicidal effect on the target microorganism and to preserve the properties of the medium being treated, the medium should be subjected to a minimum lethal UV-C dose. The resistance of the microorganism in question to the minimum lethal UV-C dose should be carefully considered, as various factors will determine the microorganisms' resistance to the effect of UV radiation. These factors include: the species and strain of the microorganism, the growth media of the microorganism, stage of proliferation of the microorganism and the density or concentration of microorganisms, amongst others (Chang *et al.*, 1985; Wright *et al.*, 2000).

Wilson & Droby (2001) reported that the lethal UV-C dose will be higher in anaerobic conditions when compared to aerobic conditions, due to the reduced rate of oxidative reactions in anaerobic conditions. Germicidal efficacy is also negatively impacted when the water activity is decreased, or the moisture content is lowered (the liquid being treated is more concentrated). Instead of allowing a lower dose of radiation, it protects the organisms because free radicals cannot form (Wilson & Droby, 2001).

The growth mediums in which the organisms are suspended also affect the rate killing induced by UV radiation. Mediums rich in organic compounds, such as peptone, provide a protective effect against UV radiation. The growth mediums are also associated with an increase in ribosomes in the intracellular matrix of the microorganisms, which could potentially provide a shielding effect against UV radiation (Snowball & Hornsey, 1988; Tran & Farid, 2004). The stage of growth cycle of the organism is also important as UV radiation would be more effective when applied during the lag phase of growth, rather than during the logarithmic growth phase of the organisms, as reported on *E.coli* by Snowball & Hornsey (1988). Furthermore the rate of dimer formation between thymine nucleosides occurs more

rapidly at lower temperatures (i.e. temperatures below 25°C), when compared to higher temperatures. Severin *et al.* (1983), reported that the UV dose rate needed to be increased for the inactivation of *E.coli* using UV radiation, and it was concluded that it is as a result of the natural state of the single-stranded DNA at lower temperatures that affords better dimerization through UV radiation.

Table 2.3 summarizes the results of various UV disinfection studies and shows acceptable ranges for average D-values of various microorganisms (Koutchma, 2009). Yeast and moulds are generally more resistant to UV radiation when compared to other microorganisms, possibly due to its difference in DNA structure and the larger size of the organisms (Hansen, 1976; Montgomery, 1985). Generally yeasts and moulds have less pyrimidine base pairs (principally thymine) in their DNA structure and a different chemical composition and thickness of the cell walls when compared to bacteria (Tran & Farid, 2004). The susceptibility to UV radiation can differ between different species, illustrated when comparing *Alternaria alternata* and *Aspergillus carbonarius*, both of whom are very resistant to UV radiation, with *Penicillium janthinellum*, which is very susceptible to radiation (Kyzlink, 1990).

Table 2.3: UV inactivation dose (mJ.cm^{-2}) measured at 253.7 nm for various microorganisms (Koutchma, 2009).

Microorganism	D-Value (mJ.cm^{-2})
Enteral bacteria	2 – 8
Cocci and micrococci	1.5 – 20
Spore formers	4 – 30
Enteric formers	5 – 30
Yeasts	2.3 – 8
Moulds	30 – 300
Protozoa	60 – 120
Algae	300 - 600

As for bacteria, in general bacterial spores are the more resistant to UV radiation, followed by Gram-positive and Gram-negative bacteria. The reason for Gram-positive bacteria being more resistant than Gram-negative bacteria can possibly be linked to the difference in the bacterial cell wall's structure. The teichoic acids in the Gram-positive peptidoglycan cell membranes are extremely rigid, and because Gram-

negative bacteria do not have such teichoic acids, they are less rigid and therefore more susceptible to UV radiation. Furthermore small coccoid bacteria are usually more resistant than rod-shaped bacteria as the surface-to-volume ratio of the coccoid bacteria allows less absorption of UV radiation in the cellular matrix, compared to rod-shaped bacteria (Lado & Yousef, 2002). Coccoid organisms, such as *Micrococcus radiodurans* and *Micrococcus radiophilus*, have the ability to repair radiation damaged DNA due to high catalase activity (Kyzlink, 1990). The catalase activity has been implicated in the alleviation of the effects of free radicals formed by UV radiation. The fluidity of an organism's membrane also plays a significant role in the susceptibility of that organism to UV radiation. A higher fluidity increases resistance to radiation, due to the high concentration of unsaturated fatty acids (Lado & Yousef, 2002).

Gram-positive organisms that are more sensitive to UV radiation include the genera *Leuconostoc*, *Lactobacillus* and *Staphylococcus* in particular *Staphylococcus aureus* and *Staphylococcus faecalis* (Kyzlink, 1990; Zadoks *et al.*, 2001)). The most sensitive bacteria include the Gram-negative enterobacteria, especially the members of the genera *Escherichia*, *Enterobacter* and *Salmonella*. The genera *Proteus* and *Pseudomonas* have variable levels of UV resistance (Kyzlink, 1990). Bacterial spores are normally more resistant than the living forms of the organism and non-sporulating microbes. The magnitude of the lethal dose is correlated to the low content of -SH groups in the spores. The lethal dose also depends on the acidity of the environment. Among the spore-formers, *Clostridium botulinum* and *Clostridium sporogenes* are some of the more resistant organisms, while spores of the thermophilic types of organisms, which are responsible for souring of milk, are much more sensitive (Kyzlink, 1990).

Viruses also deserve mention, as they cause many food-borne illnesses. Viruses in general are very resistant to radiation (Kyzlink, 1990, Koutchma, 2009).

2.4.2. Process characteristics

Process parameters and processing characteristics would greatly determine the germicidal efficacy of UV radiation when treating liquids. When evaluating UV dose responses and designing a system to optimally treat such liquids, various intrinsic design factors should be considered.

2.4.2.1. *Source of UV radiation*

Various UV light sources are available commercially and therefore the choice of UV source will depend on factors such as the type of application, target microorganism to be treated, UV dose and wavelength needed as well as integration within the UV system design and other practical and economic considerations. The correct UV source will increase efficiency of the application and should promote the UV dose response by increasing penetration and energy transfer in the liquid being treated. It should be considered that the choice of UV source would therefore be situation-specific and that no single lamp technology at present could be regarded as the best source of UV for all food applications. Monochromatic (narrow band) and polychromatic (broadband) light sources are available offering a broad spectrum of potential applications. Various UV sources are available at present, including but not limited to the following: Mercury Lamps (low and medium pressure), Amalgam Lamps, Excimer Lamps, Broadband-Pulsed Lamps, Microwave UV Lamps and LED's. Factors that will affect the lamp performance and that should be considered when choosing the correct lamp for the application in question, include: Expected life of the lamp, lamp output and lamp output over lamp life, the temperature of operation of the lamp, reflection, scattering refraction within the system, absorption values required and other general maintenance considerations, such as power supply and cost of the lamps to be used (Guerrero-Beltrán & Barbosa-Cánovas, 2004; Masschelein, 2002; Koutchma 2009; Koutchma *et al.*, 2009).

Monochromatic low pressure mercury lamps (LPM) offers exceptional efficiency as up to 40% of the electrical power supplied to the lamp is converted to UV-C radiation energy at 253.7 nm, which represents greater than 85% of the UV intensity generated, that can be used for disinfection. The LPM lamps are extensively used in the

disinfection applications within water, air, surfaces and the food and beverage industries as well as water sewage treatment plants (Koutchma, 2009; Masschelein, 2002). Important critical control points when evaluating LPM efficacy is monitoring lamp wall temperature changes. The mercury vapour pressure in these lamps is important and as the temperature increases, UV-C output is maximised at some point. When the temperature exceeds the optimal mercury vapour pressure point, the UV wave length shifts to higher wave lengths, such as 265 nm. The wall temperature of the lamps therefore need to be carefully monitored as it is an indication of vapour pressure and ultimately UV-C output and application efficacy. Some indicators relating to the lamp life to consider include the Voltage input to the lamp, solarisation of glass and fouling on quartz sleeve within the system.

Polychromatic medium pressure mercury (MPM) lamps have a broad peak line spectrum in both the UV and visible light spectrum (250 – 600 nm) and are suitable for UV disinfection and oxidation (Koutchma, 2009; Masschelein, 2002). Typical application areas include the treatment and disinfection of larger volumes of liquids, such as waste water and drinking water, in industrial and municipal plants. It is also used for photochemistry to activate oxidation.

Low pressure mercury amalgam (LPMHO) lamps are high powered and can deliver up to 10 times the UV power density when compared to traditional low pressure mercury lamps. One of the main advantages of the LPMHO lamps is temperature tolerance, even at temperatures as high as 90°C, the UV output of the lamps will remain constant. Furthermore the coating properties of the lamps limit UV transmission loss and up to 90% of the UV-C output is still maintained at the end of the lamp life (estimated between 15,000 – 20,000 hours depending on the make and model). They are generally considered in UV applications as a cost-effective alternative to LPM lamps (Koutchma, 2009).

Excimer lamps (EL) are monochromatic UV lamps containing no mercury. EL produces no infrared radiation, therefore no heat is generated and due to the narrow band of UV radiation (single spectral line), specific wavelengths can be considered for the application of choice (Koutchma, 2009). Main areas of use of EL include medical, chemical and biological applications.

Broad pulsed lamps' emission is similar in wavelength composition to that of solar light, and although the penetration rate in turbid liquids are more efficient than that of LPM, the technology has not yet been well established in the treatment of liquids (Schaefer *et al.*, 2007).

Microwave UV lamps eliminates the need for electrodes due to being generated through a magnetron. The main advantages include a reduced deterioration during start-up when product is treated as well as instant warming-up of the lamps. Microwave UV operates at similar pressures and temperatures as LPM lamps (Meier *et al.*, 2007).

2.4.2.2. *Flow dynamics*

The flow dynamics of the UV operating system is a critical parameter in promoting effective UV-C transfer to the liquid being treated. Various reports and studies have been conducted on different UV systems and UV system configurations. Generally the UV reactor system designs can be divided into three distinct groups, namely laminar flow, turbulent flow and dean flow reactors (Koutchma, 2009). A desirable design for the UV reactors will create an ideal flow to maximize the UV dose effect. However, UV system designs could present challenges, such as dead spaces in the reactors that could negatively impact on residence time and tracer analysis can be used in such instances to determine the residence time distribution (Kessler, 2002; Koutchma & Parisi, 2004; Koutchma, 2009). The type of liquid and specifically the product viscosity would determine the choice of flow and resulting reactor design needed for a particular application.

When liquid flows through pipes and equipment, pressure losses will occur as a result of friction between the liquid and the boundary walls, the change of direction of flow and cross sectional areas. The volume flow and the velocity distribution are the two main factors that will influence such pressure losses. Laminar flow is when the flow will follow a parabolic velocity flow distribution and zero flow next to the wall of the pipe due to friction or adhesion. As flow rate (velocity) is increased, the velocity flow

distribution is altered and transverse motion will occur, hence forcing the flow to become ‘squarer’ (Fellows, 2000; Kessler, 2002; Koutchma & Parisi, 2004; Koutchma, 2009). There will be an decrease in the thickness of the boundary layer as the fluid separates from the wall, resulting in turbulent flow as indicated in Figure 2.6.

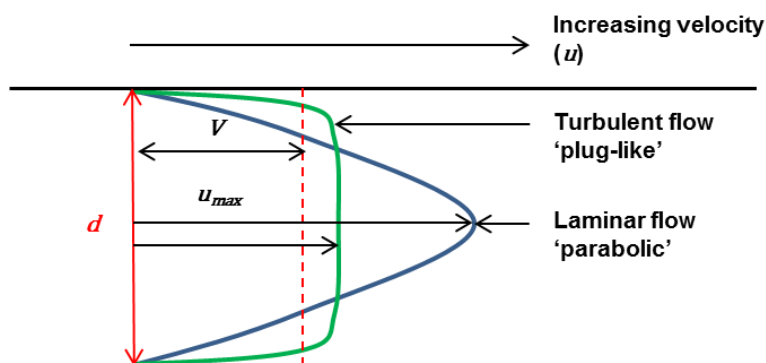


Figure 2.6: Velocity distribution to characterize the flow type where d = pipe diameter and u = velocity with u_{max} being the maximum velocity.

The Reynolds value (Re) is a number that will determine whether the flow in a pipe is laminar, transient (state between laminar and turbulent) or turbulent. If the $Re < 2300$ the flow is laminar, a Re between 2300 to 4000 is transient and $Re > 4000$ it is regarded as turbulent (Kessler, 2002). The following equation is used to calculate the Reynolds value (Fellows, 2000):

Equation 6

$$Re = \frac{\rho \cdot V_e \cdot D}{\mu}$$

where Re is the Reynolds value, ρ is the density ($\text{kg} \cdot \text{L}^{-1}$) of the liquid being treated, V_e is the flow velocity ($\text{m} \cdot \text{s}^{-1}$), D is the length (m) of the flow-path (characteristic linear dimension) and μ is the dynamic stress viscosity of the liquid (in cP or mPa-s).

In order to maximize the UV-dose response, the type of flow will determine the system design parameters. Laminar flow UV-systems in general uses extremely thin film flow chamber designs to maximize UV absorbency as the difference in the parabolic flow velocity produces non-uniform conditions that need to be exploited (Koutchma & Parisi, 2004). Examples of such commercial systems include the

CiderSure (FPE Inc., Macedon, NY, USA) and the Taylor-Couette flow UV systems (Forney *et al.*, 2004). Turbulent flow systems usually accommodates higher flow rates which makes such systems commercially more viable within a processing environment, and the turbulent conditions improve the homogeneity of the liquid being exposed as well as improve exposure and energy transfer due to better mixing. However, due to high flow rate and turbulent flow patterns, pressure drops could occur as a result of reduced fluid resistance time and increased transverse motion, which could complicate scaling-up of turbulent-flow UV systems (Simmons *et al.*, 2012). The pressure drop within such a UV-reactor is one of the main design features of the SurePure system, which addresses this ‘defect’ through a unique design feature which was one of the main reasons for patenting the SurePure TurbulatorTM (more reference to this will follow in Chapter 3, which exclusively deals with the SurePure thin-film TurbulatorTM or ‘reactor’ design). Other turbulent-flow examples include CiderSure 1500 (FPE Inc.) and the ‘Aquionics’ UV system (Hanovia Ltd., Slough England, UK) (Koutchma, 2007; Koutchma, 2009).

The Dean flow effect was first described by Dean (1927) and promotes turbulent flow, uniform velocity, residence time distribution and secondary eddy flow effect. Koutchma (2009) gives a comprehensive review and examples of Dean flow UV systems, including the coiled UV 420 model (Salcor Inc., Fallbrook, CA, USA) (Koutchma, 2007).

2.4.2.3. *Geometric configuration*

Various authors have made reference to the geometric configuration of the UV system in order to achieve desired germicidal effect when treating liquids. Donaghy *et al.* (2009) reported even if all the other factors within a system are the same, the resultant lethality of the process will be different if the geometric configuration of the UV system is changed. The FDA also refers to the geometric configuration to be an important parameter when referencing the kinetics of microbial inactivation for alternative processing technologies (Anonymous, 2012).

In essence, according to the authors' definition, the geometric configuration of UV radiation as an alternative technology to thermal pasteurisation encompasses all the design elements of the applicable system to deliver a desired germicidal efficacy of a target organism or population taking into account the intrinsic factors (product characteristics) of the product being treated (Fellows, 2000; Koutchma, 2007; Koutchma, 2009).

2.4.3. Product characteristics

The product characteristics need to be well defined and assessed in order to predict and ensure an effective UV dose response following UV radiation. Liquids have a diverse range of physical, chemical and optical properties, the latter especially relevant when assessing the use of UV technology in the treating of liquids as it is directly related to UV light transmission and germicidal efficacy. Physical properties include the viscosity and density of the liquid being treated, and whether the liquid in question would be regarded as Newtonian or Non-Newtonian. The chemical and biochemical composition would include the dissolved solids including macronutrients (such as fat, proteins and carbohydrates) and micronutrients (such as vitamin C and D) as well as organic compounds and enzymes that could ultimately influence the germicidal efficacy of UV radiation. In addition other chemical parameters that will affect UV efficacy possibly include insoluble solids, pH and the redox potential of the liquid being treated.

Table 2.4 summarizes the main results of UV radiation on various different liquids (excluding water) with different product characteristics as reported by various authors. The efficacy of UV against numerous microorganisms in drinking water is well known, and therefore was omitted from Table 2.4 (Parrotta & Bekdash, 1998).

2.4.3.1. Optical properties

The optical properties are determined by various intrinsic factors of the liquid, most notably the % soluble solids, % insoluble solids and the nutrient composition. When utilizing photochemistry, such as UV radiation, two critical conditions need to be taken into consideration according to Koutchma *et al.* (2009): First, the photons delivered to the liquid must be sufficient to induce a reaction, such as to form or break chemical bonds and second, the photon energy needs to be absorbed in order to promote reactions. In Table 2.5 representative values of typical bond energies of biological moieties are given, and from this data it is evident that wavelengths less than 320 nm are efficient in radiation energy to induce or promote a photochemical reaction.

Furthermore the absorption coefficients and resulting % UV transmittance (UVT) varies between different liquids, and is dependent on optical density influenced by the fundamental principles of light propagation as described in Figure 2.2 in section 2.2. In Table 2.6 absorption coefficients and resulting transmittance values are indicated, which confirms the exponential function of the Beer-Lambert law. As per the equation in section 2.3.5., changes in path length (x) with a high absorption coefficient (α) drastically change the % UV transmittance measured at 253.7 nm.

Table 2.4: Summary of UV-radiation germicidal efficacy as reported by various authors on different liquids being treated.

Liquid	UV-Equipment	Organisms	UV Dosage	Log reduction (cfu.mL ⁻¹)	Flow-rate	Author
Orange Juice	Falling film	TMA	214.2 W.cm ⁻²	2.673	NS*	Harrington and Hills, (1968)
Apple Cider	Thin film, laminar UV disinfection unit (Cidersure 3,500)	<i>E.coli</i> (ATCC 25922)	NS*	5 - 6	~ 6 l.min ⁻¹	Worboro <i>et al.</i> , (1999)
Apple Cider	Thin film, laminar UV disinfection unit (CIDER 10UV)	<i>E.coli</i> 0157:H7 (5 different strains)	9 – 61 mJ.cm ⁻²	3.81	0.999 – 6.48 l.min ⁻¹	Wright <i>et al.</i> , (2000)
Apple Cider	Thin film, laminar UV disinfection unit (Cidersure 3,500)	<i>Cryptosporidium parvum</i> oocyst	14.3 mJ.cm ⁻²	5	~ 6 l.min ⁻¹	Hanes <i>et al.</i> , (2002)
Apple Cider	Thin film UV disinfection unit (Cidersure 3,500)	<i>E.coli</i> 0157:H7 933 ATCC 43889 ATCC 43895	14 mJ.cm ⁻²	5.25 – 7.19 5.93 6.63 5.93	~ 6 l.min ⁻¹	Basaran <i>et al.</i> , (2004)
Apple Cider	Thin film UV disinfection unit (Cidersure 3,500)	<i>E.coli</i> ATCC 25922	> 6,500 μ W sec.cm ⁻²	5.0 – 6.0	~ 6 l.min ⁻¹	Quintero-Ramos <i>et al.</i> , (2004)

Table 2.4 (*continued*): Summary of UV-radiation germicidal efficacy as reported by various authors on different liquids being treated.

Liquid	UV-Equipment	Organisms	UV Dosage	Log reduction (cfu.mL ⁻¹)	Flow-rate	Author
Orange Juice	Thin Film, laminar	TMA and Yeast and Moulds	87 and 119 mJ.cm ⁻² respectively	1.0	0.6 l.min ⁻¹	Tran and Farid, (2004)
Apple Juice	Laminar	<i>E. coli</i> K12 ATCC 25253	14.5 mJ.cm ⁻²	3.0 – 4.0	56.8 - 165.7 mL.sec ⁻¹	Koutchma <i>et al.</i> , (2004)
Apple Cider	Turbulent	<i>E. coli</i> K12 ATCC 25922	0.75 mJ.cm ⁻²	< 1.0	250 -1300 mL.sec ⁻¹	Koutchma <i>et al.</i> , (2004)
Apple Juice	Taylor-Couette Flow	<i>E. coli</i> ATCC 15597	9 mJ.cm ⁻²	3.0 – 5.0	NS*	Forney <i>et al.</i> , (2004)
Apple Cider	Coiled Tube	<i>E. coli</i> and <i>L. innocua</i>	34 J.mL ⁻¹	4.3 2.5	27 – 83 mL.min ⁻¹	Geveke, (2005)
Apple Juice	Laminar	<i>S. cerevisiae</i> , <i>L. innocua</i> (ATCC 51742) and <i>E. coli</i> (ATCC 11775)	45 mJ.cm ⁻²	1.34 4.29 5.10	0.073– 0.548 l.min ⁻¹	Guerrero-Beltrán & Barbosa-Cánovas, (2006)
Mango Nectar	Laminar	<i>S. cerevisiae</i> , APC and Yeasts and Moulds	5,135 mJ.cm ⁻²	2.71 2.94	0.073 to 0.451 l.min ⁻¹	Guerrero-Beltrán & Barbosa-Cánovas, (2006)
Orange Juice	UV reactor (Gentra Stock Joint Co., Istanbul, turkey)	APC Yeast and Moulds <i>E.coli</i> (ATCC 25922)	36 kJ.L ⁻¹	2.80 0.34 5.72	0.02 l.sec ⁻¹	Pala & Toklucu, (2013)
Apple, Guava + Pineapple Juice, Orange Juice	Continuous, thin film turbulent flow UV system	APC, Yeasts and Moulds	229.5 J.L ⁻¹ 1,377 J.L ⁻¹ 167 J.L ⁻¹	3.5 (APC) 3.0 - 4.48 < 1.00 – 1.32	3800 L.h ⁻¹	Keyser <i>et al.</i> , (2008)
Tropical Juices Orange Juice Guava Juice Carrot Juice Pineapple Juice	Coiled tubing UV unit	<i>E. coli</i> APC Yeasts Moulds Moulds	21.5 mJ.cm ⁻²	6 1.5 1.2 3.2 1.0	NS*	Koutchma <i>et al.</i> , (2007)
Whole milk – Goat's (Skimmed, low-fat and full cream)	Thin film UV disinfection unit (Cidersure 3,500) Laminar-flow (<i>R_e</i> at < 1371)	<i>E. coli</i> ATCC 25922	5.3 – 6.3 mJ.cm ⁻²	1.44 – 2.27	151 L.h ⁻¹	Matak, (2004)
Whole milk – Goat's	Thin film UV disinfection unit (Cidersure 3,500)	<i>Listeria monocytogenes</i>	15 mJ.cm ⁻²	5.0	567 L.h ⁻¹	Matak <i>et al.</i> , (2005)
Whole milk – Cow's	Continuous, thin film turbulent flow UV system	Mesophilic aerobic bacteria	1.5 kJ.L ⁻¹	3.0	4000 L.h ⁻¹	Reinemann <i>et al.</i> , (2006)
Whole and semi-skimmed milk	Laboratory scale UV system (flow-tube with static mixers)	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>	1,000 mJ.mL ⁻¹	0.5 – 1.0	168 mL.min ⁻¹	Altic <i>et al.</i> , (2007)
Whole milk	Continuous, thin film turbulent flow UV system	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> (ATCC 43012 and ATCC 806R)	1,000 J.L ⁻¹	0.10 – 0.60	3,800 L.h ⁻¹	Donaghy <i>et al.</i> , (2009)
Raw cow's milk and skimmed milk	Coiled tube design UV reactor (Dean Flow)	<i>Escherichia coli</i> W1485 <i>Bacillus cereus</i> endospores	11.187 mJ.cm ⁻²	4.1 - 7.8 2.65 - 2.72	25 – 200 mL.min ⁻¹	Choudhary <i>et al.</i> , (2011)
Low-fat and full cream milk – Cow's	Continuous, thin film turbulent flow UV system	Various (pathogens and sporeformers)	0.334 – 1.430 kJ.L ⁻¹	1.0 (D-Value)	4000 L.h ⁻¹	Rossitto <i>et al.</i> , (2012)

* NS – Not Specified

Table 2.5: Typical Bond energies for common bonds of molecular groups of important biological moieties and their corresponding absorption wavelengths (Blatchley & Peel, 2001).

Bond	Typical Bond Energy (kJ.mole ⁻¹)	Corresponding wavelength (nm)
O-H	460	260
C-H	410	290
N-H	390	310
C-O	370	320
C=C	830	140
C=N	850	140
C=O	740	160
C≡N	600	200

Table 2.6: Absorption coefficients (α) and % transmittance values at 253.7 nm for some liquids (Koutchma *et al.* 2009).

Liquid	Absorption coefficients (α), cm ⁻¹	Transmittance, 1 cm, %	Transmittance, 1 cm, %	Penetration for 90% absorption, cm
Water	0.01	97.72	99.77	100
Waste Water	14	~ 0	3.98	0.070
Clear Apple Juice	15	~ 0	3.16	0.067
Apple Cider	40	~ 0	0.01	0.025
Orange Juice	100	~ 0	0.00	0.010
Liquid sucrose	4.5	0.0032	35.48	0.022
Beer	16	~ 0	2.51	0.063
Coca Cola	31	~ 0	0.08	0.032
Raw Milk	290	~ 0	0.00	0.003
Egg White	104	~ 0	~ 0	0.001
Sherry	9	~ 0	12.59	0.111

2.4.3.2. Physical properties

The viscosity of the liquid being treated will impact the flow dynamics of the liquid and therefore is an important parameter when specifying the design elements of the UV system. The Reynolds value (Re), which indicates laminar or turbulent flow patterns, is directly correlated to the dynamic viscosity (μ), which is measured at a

specified temperature, usually 20°C, and is recorded as centipoise (cP) or millipascal-second (mPa-s). The dynamic viscosity is defined as the resistance of a liquid to shearing flows. Although Hijnen *et al.* (2006) concluded that the UV dose response is independent of factors such as temperature, pH and the organic matter; the physical properties of liquids will change with a change in temperature. The temperature of the liquid treated will have an effect on the density (ρ), the dynamic viscosity (μ) and ultimately the R_e of the liquid, and as a result will impact its physical properties. Therefore it is important to take into consideration the effect of temperature when specifying processing parameters and design elements of the UV system used, as adjustments could be made to compensate for the change in the physical properties as a result of temperature changes in the liquid being treated. If the viscosity of the liquid being treated is independent of shear stress, the liquid will be classified as Newtonian (for example water). However, if the liquid treated does alter the viscosity as a result of shear stress, it will be classified as non-Newtonian (for example shear thinning liquids, shear thickening liquids, thixotropic liquids) (Kessler, 2002). Koutchma (2009) achieved a 5-log reduction with a standard turbulent flow number on juices (Newtonian liquids) with an absorption coefficient of less than 15 cm⁻¹. However, more viscous juices subjected to the same conditions resulted in a less than a 1-log reduction only on APC, which was attributed to the non-Newtonian nature of the higher viscosity fruit juices and altered (less efficient) flow dynamics within the UV system used (Koutchma *et al.*, 2007).

2.4.3.3. *Chemical and Biochemical properties*

The effect of UV radiation on the microbiological population within different liquids has received much attention in research, as the primary goal is to achieve germicidal efficacy upon the treatment of liquids with UV radiation. However, limited research has been conducted on the effect of UV treatment on the chemical and biochemical composition of liquids. In the instance that some studies have investigated such impact, the studies were mainly limited to water, fruit juices, sugar concentrates and goat's milk (as summarized in Table 2.4).

Total Solids (Soluble and Insoluble)

The dissolved organic solutes in liquids will have a strong UV attenuating effect (Koutchma, 2009). Suspended solids will not only attenuate the UV dose, but could also provide a ‘shielding’ effect towards microorganisms, as the microorganism could aggregate on the surfaces of suspended particles that could absorb, reflect, refract and scatter the UV light (Christenen & Linden, 2001). Although limited research has been conducted on the effect of suspended solids, Koutchma (2009) states that the UV dose delivered may be underestimated as a result of fluids with a high concentration of suspended solids thus negatively impacting the UV dose response through lower UV absorbance to the targeted microorganisms. From this it was concluded that a thorough investigation and analysis of the liquid should be concluded in order to equate UV absorbance needed for an optimal UV dose response.

Macronutrients

Foods vary enormously in their sensitivity to UV light. Degradation of macronutrients in food following exposure to sunlight or to UV radiation has been investigated since the early 1950’s. Spikes (1981) reported that unsaturated fatty acid residues in oils, solid fats, and phospholipids are usually regarded as light “light sensitive”. Degradation of especially fat and protein in foodstuffs due to light exposure occurs in the UV and visible light region of the spectrum (between 280 nm & 780 nm), but the blue to green band of the visible region (430-460 nm) is considered the main band responsible for light-induced oxidation (Borle *et al.*, 2001). Koutchma (2009) indicates nucleic acids are the strongest light absorbers at the germicidal wavelength of 254.7 nm, and states that unsaturated organic molecules usually absorb at wavelengths greater than 220 nm. Carbohydrates were also indicated not to be especially sensitive to light degradation (Koutchma, 2009), however, Grandison (2006) reports that UV radiation of carbohydrate rich substances results in loss of texture or viscosity due to the breakdown of the glycosidic bond in starch, pectin or cellulose to give smaller carbohydrates.

Some defects that can occur as a result of photooxidation of fat, protein and carbohydrates include altering the nutritional profile of the food being irradiated, the development of off-flavours and aromas and changes to the rheological properties of

the food (texture and appearance). A more detailed review is given in section 2.5. with specific emphasis on the effect of UV radiation on milk, as a model liquid.

Redox potential

The UV dose response can also be subject to the redox potential of the liquid being treated and in some instances the germicidal efficacy can be enhanced when using UV in combination with other oxidizing agents such hydrogen peroxide (H₂O₂) and ozone (Blank & Cumming, 2001). The use H₂O₂ of in combination with UV is well-known in the aseptic packaging and wastewater industries, as the free radical formation of H₂O₂ and resulting germicidal efficacy is improved with UV radiation (Warriner *et al.*, 2000; Thiruvengkatachari *et al.*, 2006).

pH

Various authors reported that the pH of the medium treated does not influence the killing efficacy of the target organism (Ngadi *et al.*, 2003; Koutchma *et al.*, 2004; Murakami *et al.*, 2006; Hijnen *et al.*, 2006). However, the physical properties of the liquids in question as per their reports were not altered and in more complex liquid systems, such as milk, a change in pH could induce structural changes in organic compounds, such as protein. These changes could therefore alter the physical and biochemical properties of the liquid and could impact on the UV dose response due to a change in UV absorbance needed to have the desired effect. Therefore the effect of UV on the product characteristics of more complex biological liquids, such as milk, remains of interest.

Vitamins

Bekbölet (1990) stated that off-flavours and odours introduced following photooxidation are directly related to the source of light, the radiation dosage, the type of liquid being treated, the temperature of the liquid, the presence of oxygen and wavelength; however other constituents responsible for photochemical changes include proteins, amino acids, riboflavin, and vitamins. According to Grandison (2006), the destruction of vitamins is dependent on the chemical structure of the vitamin itself.

Various researchers have shown that exposure to visible light between 365 and 500 nm causes a significant increase in light oxidation in milk (Bosset *et al.*, 1995; Hansen & Skibsted, 2000; Lennersten & Lingnert, 2000; Van Aardt *et al.*, 2001). Riboflavin (Vitamin B2) is implicated in this oxidation because it acts as a photosensitizer when exposed to specific wavelengths (400, 446, and 570 nm) within this range (Lee, 2002; Bekbölet, 1990). Other water-soluble vitamins susceptible to photooxidation include Thiamine (vitamin B1) and Ascorbic acid (vitamin C). Wilson & Droby (2001) reported that ascorbic acid in fruit juice was converted to dehydro-ascorbic acid, the oxidized form, which is biologically active and converted back to the reduced form during storage. The order of sensitivity of the water-soluble vitamins to photooxidation is as follows: thiamin > ascorbic acid > pyridoxine > riboflavin > folic acid > cobalamin > nicotinic acid (Fellows, 2000). The fat-soluble vitamins vary in their sensitivity to radiation, with Vitamins D and K largely unaffected while vitamins A and E are sensitive to photooxidation. The order of sensitivity of the fat-soluble group of vitamins is: vitamin E > vitamin A > vitamin K > vitamin D (Fellows, 2000). To minimize the loss of vitamins, liquids should be packaged in the absence of oxygen and irradiated at low temperatures with further losses that can be prevented by storing the irradiated products at low temperatures (Wilson & Droby, 2001).

Furan

It is well-known that furan is formed in food and drinks during thermal processing, and is present in foods such as coffee and processed baby foods (Moro *et al.*, 2012). Furan derivatives, particularly furfural and hydroxymethylfurfural, are major volatile constituents of heated milk (Burton, 1988). Furans are not found in raw milk, but appear in milk heated above 90°C due to the Maillard reaction (Ferretti *et al.*, 1971; Shibamoto, 1980). Furan has been reported as a possible human carcinogen, and therefore is undesirable in any consumed food or drinks (Bakhiya & Appel, 2010). In a study by Fan & Geveke (2007) they showed that UV-C treatment of apple cider caused the formation of furan at high doses of radiation. It was concluded that fructose was probably the main source of furan following UV radiation, however, it was also shown that very low amounts of furan were formed at the UV-C dose that was required for the inactivation of *E. coli* K12.

Cholesterol and cholesterol oxides

Cholesterol is the main sterol in animal-derived products, such as milk. It is well-known that cholesterol, like other sterols, are susceptible to oxidation by reactive oxygen species, light, UV light, ionizing radiation, chemical catalysts, lipid hydroxyperoxides, and enzymatic reactions, leading to the formation of sterol oxidation products (SOPs) (Sieber, 2005). Furthermore, human studies reveal that cholesterol oxidation products (COPs) could be absorbed from the diet (Linseisen & Wolfram, 1998).

2.5. Ultraviolet radiation and its application in the dairy industry

Milk and dairy products have always provided a model system for scientific research for a number of reasons. Milk is a nutrient rich product with a high nutritional value which contains lipids (which include essential fatty acids), proteins and peptides, essential amino acids, immunoglobulins, enzymes, enzyme inhibitors, growth factors, hormones and antibacterial agents, lactose, vitamins and inorganic elements such as calcium and water (Fox & McSweeney, 1998; Matak, 2004). Furthermore milk is regarded as a complex biological system, as it also contains various microorganisms as well as somatic cells (white blood cells) (Ruegg & Reinemann, 2002).

Milk and dairy products play an important role in the agricultural industrial sector and economy globally. In 2011 global milk production was estimated at 748 million metric tons (MT) of which cow's milk contributed approximately 621 million MT, produced by an estimated 260 million cows (OECD-FAO, 2012). The most recent data confirms the gross production value of agriculture at USD 3.282 billion, whereas raw milk production worldwide currently represents almost 8.9 % of the total agricultural gross production value, estimated at USD 292 billion. The trade of dairy products, including cheese and milk powder, represents 5.9 % of all agricultural products trade globally, with a total estimated value of USD 64 billion (IDF, 2014). In addition to the current production value of milk and dairy products, milk production globally is increasing by approximately 2.2% per annum. Consumer demand is also increasing, especially in developing countries, and a greater than 20% increase of milk and dairy products consumption is expected by 2021 (OECD-FAO,

2012; IDF 2014). An estimated 750 million people are engaged in milk production globally and it is estimated that for every 100 litres of milk produced, at least 5 jobs are created. In South Africa approximately 3% of the agricultural labour force works in the dairy sector, which is below average when compared to the rest of the world where employment provided by the dairy sector could be as high as 30% of the total agricultural labour force in countries like Switzerland, Norway and Argentina (IDF, 2014).

2.5.1. Bovine milk

Bovine milk can be defined as the unadulterated, fresh liquid that is expressed from the udder of cows when milked. This, however, excludes the liquid expressed 15 days before and 5 days after calving, this is known as colostrum. Good quality raw milk is required to make good quality dairy products. Once raw milk is defective and of inferior quality, it cannot be improved during processing, and defects often become more pronounced.

2.5.1.1. *Microbiology of raw milk*

Milk from healthy cows contains relatively few bacteria, between 100 –1,000 cfu.mL⁻¹, thus the risk from drinking raw milk following good manufacturing and hygiene practices, would be minimal (Kurweil, 1973; Robinson, 2002). However, milk can be classified as a potentially hazardous food if it is not properly processed, handled, or stored as milk has limited protection from external contamination. Milk can be contaminated easily once it is separated from the cow, and the microflora present can be attributed to three main sources of contamination: Firstly, microbial contamination can originate from infection within the udder of cow. Secondly, contamination can occur from the exterior surface of the udder during milking and thirdly contamination can occur from exterior surfaces (such as milking machines, pipelines and storage containers) (Rosenthal, 1991; Robinson, 2002; Jay, 2005). The length of storage of milk before further processing as well as the temperature of storage will also influence the microbial population of milk, and therefore effective

cooling and refrigeration is essential to maintain its microbiological quality and integrity.

Natural flora originating from the cow generally has little influence on total plate counts (Murphy & Boor, 2000; Jay, 2005). Mastitis, defined as the inflammation of the mammary gland, is associated with an increase in the Somatic cell count in the milk and indicates primary infection in the udder of the cow. According to Bramley & McKinnon (1990), bacteria responsible for mastitis can be categorized as major and minor pathogens. During mastitis the total bacterial numbers can increase to as much as 10^7 cfu.mL⁻¹, and will have a significant impact on the bulk milk's total microbial population (Bramley & McKinnon, 1990; Jeffrey & Wilson, 1987; Ruegg & Reinemann, 2002; Barbano *et al.*, 2006; Ruegg, 2011). The organism responsible for infection and the stage infection will mainly determine the aerobic plate count (APC) and bacterial population within the milk. The microorganisms *Streptococcus* spp., most noticeably *Str. agalactiae* and *Str. uberis*, are usually associated with udder infection, whereas *Staphylococcus aureus* occurs less frequent during mastitic conditions (Gonzales *et al.*, 1986; Zadoks *et al.*, 2004; Barbano *et al.*, 2006; Ruegg, 2011). In general there is poor correlation with the somatic cell response and infections due to bacteria such as *Staphylococcus* spp., coliforms and streptococci. Other bacteria that have been associated with mastitis include *Leptospira* spp., *Listeria monocytogenes*, *Bacillus cereus* and *Clostridium perfringens* (IDF, 1996; Zadoks *et al.*, 2004).

Microbial contamination from exterior surfaces is mainly as a result of contamination of the milk through the surface of teats and udders. The organisms associated with exterior surface contamination include streptococci, staphylococci, spore-formers, coliforms and other Gram-negative bacteria, and when originating from the udder mainly includes micrococci and aerobic spore formers. However, both thermotolerant bacteria (that survive pasteurisation) and psychrotrophic bacteria (that grow under refrigeration) are commonly found on teat surfaces, and plays a significant role in milk composition and quality when further processing milk (McKinnon *et al.*, 1990; *et al.* 1990; Robinson, 2002; Jay, 2005). Pathogenic bacteria that might contaminate the teats include *Campylobacter jejuni*, *Salmonella typhii*, *S. dublin* and *Yersinia enterocolytica*. Preventative measures to

limit these bacterial populations in the milk, mainly include strict hygienic pre-milking udder hygiene practices (McKinnon *et al.*, 1990; Galton *et al.* 1984; Pankey, 1989; Ruegg & Reinemann, 2002; Ruegg, 2011).

Microbial contamination from milking machines, pipelines and containers relates to the degree of cleanliness of the milking system, and is regarded as one of the main factors in determining the final bulk milk bacteria count (Burton, 1986; Pantoja *et al.*, 2009). Cleaning and sanitizing procedures can influence the type of microbial growth and the degree of contamination on milk contact surfaces, therefore effective cleaning regimes need to be in place to ensure that the conditions that might select for specific microbial groups are limited as far as possible (Shapton & Shapton, 1991; Brooks & Flint, 2008). Refrigeration and storage of milk, while preventing the growth of non-psychrotroph bacteria, will change the bacterial population in milk upon storage as the growth of psychrotrophic bacteria will continue, regardless of refrigeration (Hantsis-Zacharov & Halpern, 2007; De Jonghe *et al.*, 2011).

The total microbial population that are normally associated with raw milk are usually from one of four general groups, namely lactic acid bacteria, coliforms, spoilage microorganisms, and pathogenic microorganisms (ICMSF, 1998; Robinson, 2002; Hantsis-Zacharov & Halpern, 2007; De Jonghe *et al.*, 2011). In addition, Frazier & Westhoff (1998) attributed the deterioration of milk upon storage to mainly lactic streptococci, coliforms, Gram-negative rods and thermotolerant bacteria. Microorganisms can cause spoilage of milk by degrading proteins, carbohydrates and fats and are responsible for the deterioration of milk quality, particularly through the action of Gram-negative microorganisms, as storage and holding times on the farm have increased with changes in technology, marketing conditions and consumer demand (Frank, 1997; Ma *et al.*, 2000; Jay, 2000; Zadoks *et al.*, 2004; Jay, 2005; Ray & Bhunia, 2014). In Table 2.7 the main microorganisms occurring in milk are summarized and as well as a short description on their resulting impact on milk and dairy products.

Milk is susceptible to contamination by many pathogenic microorganisms, which result in infection and could be detrimental to the consumer's health (Spreer, 1998; Jay, 2005). The main pathogenic bacteria associated with milk products are *Coxiella*

burnettii, *Brucella* spp., *Salmonella* spp., *Mycobacterium tuberculosis*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 (Frank, 1997; ICMSE, 1998; Jay, 2000; Jay, 2005; Ray & Bhunia, 2014). A summary of the main types of pathogenic microorganisms, as well as the decimal reduction for low temperature long time (LTLT) and high temperature short time (HTST) pasteurisation and z-values found in raw milk are indicated in Table 2.8.

Table 2.7: The main microorganism associated with the contamination of milk.

Organism	Description	Source of contamination	Characteristics/ Defect caused	Reference
<i>Bacillus</i> spp. <i>B. licheniformis</i> <i>B. cereus</i> <i>B. subtilis</i> <i>B. mycoides</i> <i>B. megaterium</i> <i>B. polymixa</i>	Thermodeuric, Sporeforming, Aerobic, Facultatively anaerobic, can be psychrotrophic, Gram- positive, rod shaped	Dust (hay, straw and grains, milk contact surfaces, milking equipment, flooring and drains in milking parlour and processing plant, environmental (water, soil)	Sweet curdling, bitty cream (<i>B. cereus</i>), bitter flavour in pasteurised milk, proteolytic activity (<i>B.</i> <i>cereus</i> and <i>B. subtilis</i>), gas forming (<i>B.</i> <i>polymixa</i>)	Chalmers, (1955) Hayes, (1981) Bouman <i>et al.</i> , (1982) Driessen <i>et al.</i> , (1984) Langeveld <i>et al.</i> , (1995), Hantsis-Zacharov & Halpern, (2007) De Jonghe <i>et al.</i> , (2011)
<i>Clostridium</i> spp. <i>C. butyricum</i> <i>C. sporogenes</i> <i>C. botulinum</i>	Thermodeuric, Sporeforming, Anaerobic, Gram- positive	Soil, silage, feed, manure	Inhibit growth of lactic acid bacteria, produce gas and CO ₂ , proteolytic and/or saccharolytic	Hayes, (1981) Lewis, (1983), Robinson, (2002)
<i>Streptococcus</i> spp <i>S. thermophilus</i> <i>S. uberis</i>	Thermodeuric Non-sporeforming, Gram-positive, spherical	Environment and poor hygienic practises	Gas production	Hayes, (1981) Zoon & Allersma, (1996) Robinson, (2002) Zadoks <i>et al.</i> (2004)
<i>Lactobacillus</i> spp. <i>Lb. bulgaricus</i> <i>Lb. lactis</i>	Lactic Acid Bacteria; Thermodeuric, Non-sporeforming, Facultative anaerobic, Gram-positive, rods	Environment and poor hygienic practises	Acidification, production of H ₂ , CO ₂ , ethanol, formic acid, acetic acid, lactic acid.	Frazer & Westhoff, (1998) Jay, (2005)
<i>Micrococcus</i> spp. <i>M. luteus</i>	Thermodeuric, Non-sporeforming, Psychrotrophic, Gram- positive	Environment and poor hygienic practises	Lipase, hydrolysis of milk fat to glycerol and free fatty acids, production ketones, aldehydes (off- flavours)	Burton, (1986) Jay, (2005)

Table 2.8(continued): The main microorganism associated with the contamination of milk.

Organism	Description	Source of contamination	Characteristics/ Defect caused	Reference
Microbacterium <i>Microbacterium imperiale</i> ; <i>Microbacterium lacticum</i> ; <i>Microbacterium laevaniformans</i>	Thermodeuric, Non-sporeforming, Gram-positive	Environment and milk contact surfaces, equipment, flooring and drains in milking parlour and processing plant, environmental (water, soil)	Spoilage of milk post pasteurisation; indicator of milk hygiene	Hayes, (1981) Washam <i>et al.</i> , (1977) Hantsis-Zacharov & Halpern, (2007)
Enterobacteriaceae <i>Eschericia coli</i> <i>Enterobacter aerogenes</i> <i>Klebsiella pneumoniae</i> <i>Citrobacter</i> species <i>Shigella</i>	Facultative anaerobic, Gram-negative, short rods	Environment and poor hygienic practises, soil, water, waste water	Production of gas and acid, flavour defects, human disease	IDF, (1994)
<i>Pseudomonas</i> spp. <i>P. fluorescens</i> <i>P. fragi</i>	Psychrotropic, Aerobic, Gram negative, rod-shaped	Environment and poor hygienic practises	Hydrolyse proteins and lipids, esterification of alcohols (<i>P. fragi</i>), production of fruity off-flavours	Garcia <i>et al.</i> , (1989) Griffiths <i>et al.</i> , (1987) Reddy <i>et al.</i> , (1968) De Jonghe <i>et al.</i> , (2011)
Flavobacterium	Psychrotropic, Gram-negative, rod shaped	Soil and water	Hydrolyse proteins and lipids	Muir <i>et al.</i> , (1979)
Alcaligenes <i>A. viscolactis</i> <i>A. faecalis</i>	Gram-negative, Psychrotropic, rod shaped (mainly aerobic)	Soil and water, manure	Hydrolyse proteins and lipids, ropiness in milk	Muir <i>et al.</i> , (1979), Gainor & Wegemer, (1954)
Eubacterium	Facultative anaerobic, Gram-positive or Gram-negative rods	Saliva, environmental	Gas formation in Cheddar as it can grow in NaCl concentration 9.5%	Myhr <i>et al.</i> , (1982)
Enterococcus <i>E. faecalis</i> subsp. <i>liquefaciens</i> <i>E. faecium</i> <i>E. avium</i> <i>E. faecalis</i>	Lactic Acid Bacteria, Facultative anaerobic, Gram-positive cocci	Water sources, exterior of the animal and/or of milking equipment, bulk storage tanks, faecal matter	Inhibit eye development in Swiss cheese, inhibits propionibacteria (<i>E. faecalis</i> subsp. <i>liquefaciens</i>)	Klein, (2003) Batish <i>et al.</i> , (1985)

Table 2.9: Pathogenic microorganisms of concern in raw milk. The decimal kill expected under both LTLT and HTST conditions are indicated as well as the z-values where known. The table has been completed (where possible) either by using provided z values or by linear regression of thermal inactivation data for both LTLT and HTST pasteurisation (adapted from Hudson *et al.* 2003).

Pathogen	Disease	Decimal reduction LTLT (63°C/30 min) (z-value used)	Decimal reduction HTST (72°C/15 sec) (z-value used)	Reported z-value (°C) ⁴	Estimated D-value ⁵	References
<i>Brucella</i> spp. <i>B. abortus</i> <i>B. melitensis</i>	Illness with flu-like symptoms	> 300	> 114 (4.55)	4.3-4.8, 5.3	< 6.5 sec at 63.3°C 0.1 – 0.2 at 65°C	Foster <i>et al.</i> , (1953) Kronenwett <i>et al.</i> , (1954) Meisel, <i>et al.</i> , (2012)
<i>Cryptosporidium</i> spp. <i>C. parvum</i>	Gastrointestinal illness with diarrhea	~	~	~	> 5 log reduction 5 sec at 71.7°C	Harp <i>et al.</i> , (1996)
<i>Campylobacter</i> spp. <i>C. jejuni</i>	Gastroenteritis	395.6	72.8	5.1, 5.6, 6.1	0.71-0.78 min at 56°C 0.24-0.28 58°C 0.12-0.14 at 60°C	Gill <i>et al.</i> , (1981) ICMSF, (1996) Sorqvist, (1989)
<i>Clostridium botulinum</i>	~	~	~	~	Spores: >72 X 10 ⁹ reduction 240 min at 100°C Endospores: > 60 X 10 ⁹ reduction 5 min at 120°C	Jay (1986), Jay (2005)
<i>Coxiella burnetii</i> Most heat resistant non-sporeforming pathogen	Q fever	~	~	~	> 5 log at LTLT and HTST (guinea pig inoculum) 0.5-0.6 at 65°C	Enright <i>et al.</i> , (1957) Kim <i>et al.</i> , (2005)
<i>E.coli</i> O157:H7	Hemolytic uremic syndrome (HUS)	600 ¹ 130.4 ¹ 428.6 ¹	~	~	2.8 – 13.8 sec at 63°C (apple juice) 0.13 sec at 65°C	D'Aoust <i>et al.</i> , (1988)
<i>Listeria monocytogenes</i>	Listeriosis	54 ¹ 90.5 ¹ 30-75 ¹ 33.4 ⁴ 101.1 (5.91) ²	11.5 ¹ 16.7 ¹ 20.8-8.3 (5.91) ² 3.7 ⁴ 28.1 ⁴	4.3, 4.8, 5.9, 6.0, 6.1, 6.3, 8.0 (mean 5.91)	13.4 - 33.3 sec (63.3°C) 1.3 sec (71.7°C) 0.5 – 0.9 sec (74.4°C)	Bunning <i>et al.</i> , (1988) Bradshaw <i>et al.</i> , (1985)

Table 2.8 (*continued*): Pathogenic microorganisms of concern in raw milk. The decimal kill expected under both LTLT and HTST conditions are indicated as well as the z-values where known. The table has been completed (where possible) either by using provided z values or by linear regression of thermal inactivation data for both LTLT and HTST pasteurisation (adapted from Hudson *et al.* 2003).

Pathogen	Disease	Decimal reduction LTLT (63°C/30 min) (z-value used)	Decimal reduction HTST (72°C/15 s) (z-value used)	Reported z-value (°C) ⁴	Estimated D-value ⁵	References
<i>Salmonella</i> spp.	Gastroenteritis Typhoid fever	300 ³ 272 ¹ 486-818 ¹	93.8 ³ 113.1 (5.3) ² 202-340 (5.3) ²	5.3	3.5 – 5.9 at 60°C 2.2 – 3.2 s at 66°C	D'Aoust <i>et al.</i> , (1987)
<i>Streptococcus B agalactiae</i> <i>Str. Pyogenes</i>	Bacterial septicemia (newborns)	~	~	~	2 -3 s at 61.5°C 0.33 s at 62°C 0.01 s at 72°C	ICMSF, (1996)
<i>Staphylococcus aureus</i>	Respiratory disease, skin infections, Gastroenteritis	72.4 ³	5.5 ³	9.5	0.2 min at 65°C 0.02 min at 75°C	ICMSF, (1996)
<i>Mycobacterium bovis</i> <i>M. tuberculosis</i>	Tuberculosis	>273 ¹	>144 (5.0) ²	4.8, 4.9, 5.2 (mean 5.0)	4.8 – 6.6 s at 64°C 0.4 – 0.6 s at 69°C	Kells and Lear, (1960) Robinson, (2002) Jay, (2005)
<i>Mycobacterium para tuberculosis</i>	Johne's disease (ruminants) Chrone's disease (humans) possible	< 2 - > 10 120 ¹	< 2 - > 7 ⁴ 11.1 (8.6) ²	8.6	15.0 ± 2.8 s at 63°C 5.9 ± 0.7 s at 66°C < 2.03 s at 72°C (calculated)	Collins <i>et al.</i> , (2000) Pearce <i>et al.</i> , (2001) Altic <i>et al.</i> , (2007)
<i>Yersinia enterocolitica</i>	Gastroenteritis	> 200 - >158 ¹ >5 ¹ 101-2571 ¹ 31.3-125 ¹ 117.6 ³	>96.2 - >76.0 ² (5.11) >2.4 ² (5.11) 48.6-1236.4 ² (5.11) 15.0-60.1 ² (5.11) 16.1 ³	4.0 - 4.52, 5.11-5.78, 5.3, 6.0 (mean 5.11)	0.7 – 17.8 s at 62.8°C 0.24 – 0.96 min at 62.8°C	Francis <i>et al.</i> (1980) Lovett <i>et al.</i> (1982) Jay, (2005)

¹ Calculated from D-value given for the appropriate temperature

² Calculated using z-value indicated

³ Calculated using linear regression of data points

⁴ z-value is defined as the temperature change required to alter the D-value by a factor of 10.

⁵ D-value is defined as the time at a given temperature required to reduce the population of organisms present by 90% or 1 log

Moulds are undesirable in most dairy products because they produce a musty odour, affect flavour and, when growing on the surface of a product, appear unsightly. Mould growth may be an indication of poor storage or unhygienic production of dairy products (Hayes, 1981). Yeast spoilage of milk and dairy products can also occur as a result of mainly post pasteurisation contamination through environmental factors and can produce unwanted gas formation during fermentation of fresh milk (Hayes, 1981).

2.5.1.2. *Chemical and Biochemical composition of raw milk*

Milk is a biological fluid and per basic definition is regarded as an emulsion of fat globules and a colloidal suspension/dispersion of casein micelles all suspended in the aqueous milk serum phase. The serum phase is described as a true solution containing several compounds such as lactose, organic and inorganic salts and vitamins. The colloidal chemistry of milk is important when considering surface chemistry, light scattering and rheological properties of milk when processing with UV light (Fox & McSweeney, 2006). The basic composition of raw bulk cow's milk is given in Table 2.9, and it is important to highlight that the table only represents the major components in milk as milk contains several hundred minor compounds (Fox & McSweeney, 1998; Fox *et al.*, 2004; Fox & McSweeney, 2006; Fox & McSweeney, 2009).

In 1674, Van Leeuwenhoek identified microscopic particles in milk, which was identified as fat globules in milk (Fox & McSweeney, 1998). Milk fat is an oil in water emulsion, which consists mainly of triglycerides (between 97-98% of milk fat), diglycerides and phospholipids (Fox & McSweeney, 2006). The diglycerides in milk in most instances is mainly as a result of incompletely synthesized lipids or partially hydrolysed triglycerides and represents less than 0.40% of total milk fat. Phospholipids contribute to less than 1% of the total milk fat fraction and mainly consist of phosphatidylcholine, phosphatidylethanolamine and sphingomyelin (Keenan & Patton, 1995). The principle sterol in milk is cholesterol (> 95 % of total sterols in milk) and combined with cholesterol esters, which are present in trace amounts of milk, represents less than 0.35% of the total milk fat fraction (Fox & McSweeney, 1998). Furthermore, several other hydrocarbons are present in trace amounts, most noticeably carotenes, at an estimated concentration of 200 $\mu\text{g.L}^{-1}$,

which gives milk its characteristic ‘creamy’ colour, especially in high fat milk (Fox & McSweeney, 1998; Fox & McSweeney, 2006). In ruminants a wide variety of fatty acids occur (> 400 fatty acids). Cows (ruminants) milk contains a high level of butanoic acid (C4:0) and other short chained fatty acids, which contributes to between 11 - 15% of total fatty acid composition in milk, and also gives cow’s milk its characteristic flavour and aroma. The short-chained fatty acids is important to consider during processing and manufacturing of dairy products and the concentration and type of short chained fatty acids in final products can determine its organoleptic properties and overall customer acceptability (Mulder & Walstra, 1974). Low levels of polyunsaturated fatty acids (PUFA’s) are present in cow’s milk, and in general are considered nutritionally undesirable by dairy producers. Cow’s milk is rich in medium chained fatty acids octanoic (C8:0), decanoic (C 10:0), C10:1, lauric (C12:0) and C12:1, with lauric acid (C12:0) being the most abundant (McPherson & Kitchen, 1983). More than 67% of the fatty acids in cow’s milk are long chained fatty acids with hexadecanoic acid (C16:0) at ~26%, octadecanoic (C18:0) at ~15% and cis-9-octadecenoic (C18:1) at ~30% as a % of total weight of total fatty acids of triglycerides (McPherson & Kitchen, 1983; Fox & McSweeney, 2006).

Table 2.10: Typical composition of mid-lactation cow’s bulk milk (Fox & McSweeney, 1998)

Constituent	Concentration (g.l ⁻¹)	Percentage (m/m)
Fat	37.00	3.58
Protein	34.00	3.29
Casein	27.60	2.67
α_{s1} -casein	10.54	1.02
α_{s2} -casein	2.86	0.27
β -casein ¹	10.62	1.03
κ -casein	3.58	0.35
Whey protein	6.40	0.62
β -lactoglobulin	0.61	0.06
α -lactoglobulin	0.24	0.02
Bovine serum albumin	0.09	0.01
Minor components ²	0.20	0.02
Non-protein Nitrogen	1.90	0.18
Lactose	48.00	4.64
Ash (minerals)	7.00	0.68
Total Solids	127.90	12.37

¹Including γ -casein

²Including immunoglobulins

Table 2.10 summarizes the composition of full cream milk (Whole, Fresh, UHT/Long-life) according to the data of the MRC Food Composition Tables and includes the values of the main vitamins and minerals (MRC, 2014).

Table 2.11: Whole, Fresh, UHT/Long-life milk composition according to the MRC Food Composition Tables, including the Recommended Dietary Allowance (MRC, 2014).

Nutrient	Unit of measurement	per 100 mL	% RDA* per 100 mL serving
Energy	kJ	257	**
Protein	g	3.30	5.9%
Total Carbohydrate	g	4.70	**
Total fat	g	3.30	**
<i>Saturated fat</i>	g	2.08	
<i>Monounsaturated fat</i>	g	0.96	
<i>Polyunsaturated fat</i>	g	0.12	
<i>Trans fatty acids</i>	g	< 0.10	
Cholesterol	mg	14.0	< 300 mg/day**
Dietary Fibre	g	0.0	20 - 25 g/day**
Sodium	mg	49.0	< 2000 mg/day**
Calcium	mg	119.0	10.8%
Phosphorous	mg	93.0	10.6%
Magnesium	mg	13.0	3.7%
Iron	mg	0.1	0.7%
Zinc	mg	0.4	2.5%
Vitamin A	REa	31.0	3.9%
Vitamin D	µg b	0.0	0.5%
Vitamin E	mgc	0.1	0.6%
Vitamin C	mg	1.0	1.7%
Vitamin B1 Thiamin	mg	0.0	2.9%
Vitamin B2 Riboflavin	mg	0.2	10.0%
Niacin	mg	0.1	0.6%
Vitamin B6 Pyridoxine	mg	0.0	2.2%
Folic acid	µg	5.0	1.3%
Vitamin B12	µg	0.4	16.7%
Biotin	µg	1.9	6.3%
Pantothenic acid	mg	0.3	6.2%

* RDA = Recommended Dietary Allowance for people 10 years and older based on a 10 000 kJ diet. Daily values may be higher or lower depending on your kilojoule needs (weight, height, age and sex)

** WHO Prudent Dietary Goals

Energy from Protein: 10 - 15% of total energy intake/day;

Energy from Carbohydrate: 55 - 75% of total energy intake per day;

Energy from Total fat: 15 - 30% of total energy intake per day.

^aRetinol equivalents: 1 retinol equivalent (RE) = 1 µg retinol = 3.33 I.U. (International Units) Vitamin A = 6 µg trans-b-carotene = 12 µg other provitamin A carotenoids

^bAs cholecalciferol: 1 µg cholecalciferol = 40 I.U. of vitamin D"

^cα-tocopherol equivalents: 1 mg d,α-tocopherol = 1 α-TE (tocopherol equivalents = 1.49 I.U. (1 I.U. = mg dl-α-tocopherylacetate)

Iso-electric casein constitutes over 80 % of the total protein in milk, and consists of four primary caseins, namely: α_{s1} - (α_{s1} -), α_{s2} - (α_{s2} -), beta- (β -) and kappa (κ -) casein (Fox & McSweeney, 1998). Caseins exhibit “micro-heterogeneity” due to the variations in the degree of disulphide-linked polymerization, glycosylation and genetic polymorphism (genetically controlled amino-acid distribution) (Hipp *et al.*, 1952; Waugh *et al.* 1970). The casein micelle structure in milk is porous and is composed of submicelles (McMahon & Brown, 1984; Walstra, 1999). The hydrophobic core of the submicelles consists of the calcium-sensitive α_{s1} -, α_{s2} - and β -caseins, with variable amounts of κ -casein located principally on the surface of the casein micelle. The hydrophobic N-terminal of the κ -caseins interact hydrophobically with the α_{s1} -, α_{s2} - and β -caseins, while the hydrophilic C-terminal protruding from the surface of the casein submicelles forms the “hairy” region (Fox *et al.* 2004). Casein in milk consists of physical protein which attributes to 94 % of the dry weight and the remaining 6 % consists of citrate and calcium, phosphate and magnesium ions (McMahon & Brown, 1984; Holt, 1992; De Kruif, 1998). The calcium and phosphate are referred to collectively as the colloidal calcium-phosphate fraction in the milk.

Whey proteins are typical globular proteins with high levels of secondary, tertiary and quaternary structures. The four major constituents of the whey protein fractions in milk, from healthy mid-lactation cows, are: β -lactoglobulin (50%), α -lactalbumin (20%), bovine serum albumin (BSA, 10%) and immunoglobulins (Ig, 10%) - mainly IgG₁ with lesser amounts of IgG₂, IgM and IgA present (Fox & McSweeney, 1998; Fox & McSweeney, 2003). Whey proteins are denatured when heating takes place at temperatures above 65°C, which is an important characteristic to take into consideration when manufacturing dairy products, such as cheese and yoghurt (Fox & McSweeney, 2003). The whey proteins in bovine milk contain intra-molecular disulfide bonds that stabilize their structure and are not phosphorylated and as a result are insensitive to calcium (Fox & McSweeney, 2003).

Post secretion proteolysis of the primary casein fractions by plasmin in milk forms minor protein fractions in milk. The hydrolysed casein fractions or polypeptides include gamma-caseins (γ_1 , γ_2 , γ_3) and proteose peptones (Hipp *et al.*, 1952; Waugh *et al.*, 1970). During late lactation milk or milk with mastitis (with high somatic cell

counts) increased levels of the blood-derived proteins such as bovine serum albumin and immunoglobulin may be present.

Other protein compounds in milk include ~ 30 indigenous enzymes, which are either associated with the casein micelles or are present in the serum phase of the milk (Andrews, 1983; Fox & Morrissey, 1981; Fox & McSweeney, 1998). These enzymes originate from the blood, the secretory cell cytoplasm or the fat globule membrane. Bovine milk contains several endogenous proteases; these include plasmin, plasminogen, plasminogen activators (PA), plasmin inhibitors (PI), plasminogen activator inhibitors (PAI), thrombin, cathepsin D, acid milk proteases and aminopeptidases (Grufferty & Fox, 1988). Several proteases also derive from leukocytes (somatic cells) and bacteria in milk (Shahani *et al.*, 1973; Fox & Morrissey, 1981).

The disaccharide lactose is the principle carbohydrate found in milk and is mainly influenced by the stage of lactation and the incidence of mastitis. During mastitis there will be an increase in NaCl and a depression in the secretion lactose (Fox & McSweeney, 1998). Lactose is an important factor in regulating the osmotic pressure in milk, and consists of glucose and galactose linked by β (1-4) glycosidic bond. Mutarotation of lactose occurs between the α - and β -forms until equilibrium established in solution (Fox & McSweeney, 2009). Lactulose is an epimer of lactose where the glucose moiety is isomerized to fructose, produced under alkaline conditions following severe heat treatment of milk (Andrews, 1986). The concentration of lactulose therefore is used as an index to determine the severity of heat treatment (Andrews, 1989; IDF, 1992, IDF, 1993). As lactose is a reducing sugar it will participate in the Maillard reaction (non-enzymatic browning), which is the interaction between the carbonyl group of lactose and the amino group of proteins (ϵ -amino group of lysine to form glucosamine). The resulting effect is milk browning, the development of off-flavours and a limited loss of nutritive values (due to the reaction with lysine) (Fox & McSweeney, 2009). Furthermore the Maillard reaction introduces some changes in the functionality of milk in dairy products, such as negatively impacting on the solubility of milk powders, however on the positive side it has been indicated to retard age-gelation in UHT milk and possibly has anti-oxidant properties (Fox & McSweeney, 2009).

The minerals in milk are present either in ionic form (Na and K), colloidal form (Ca, Mg, P) or as diffusible salt of Ca and Mg, which contributes to the overall acid-base balance of milk. Typical concentrations of minerals are indicated in Table 2.10, and in range between 0.70 – 0.80 % in total in milk (Fox & McSweeney, 2006).

Milk is a source of 12 water-soluble vitamins and four fat-soluble vitamins (Fox *et al.*, 2004; Drewnowski, 2011; Benoît, 2014). Typical concentrations of vitamins are indicated in Table 2.10. Vitamin D₃ (cholecalciferol) is of particular interest as UV light (280 – 320 nm) causes photoconversion of the steroid precursor of Vitamin D₃ (7-dehydroxycholesterol) to pre-Vitamin D₃. Pre-vitamin D₃ can then incur further photoconversion to tachysterol and lumisterol or can undergo temperature dependent isomerization to cholecalciferol (Vitamin D₃) (Fox & McSweeney, 2006; Fox & McSweeney, 2009; Kulie *et al.*, 2009). Vitamin D₂ (ergocalciferol) is formed following photoconversion of ergosterol, a sterol present in yeast and moulds. Vitamin D₃ plays an important physiological function in humans as it maintains plasma calcium concentration by stimulation absorption of calcium through gastrointestinal (GI) tract. In addition to Vitamin D₃, milk is also a good source of riboflavin (~ 0.17 mg per 100 g) (Fox & McSweeney, 2006; Benoît, 2014). Riboflavin can be present in the free form (60-90% of the total riboflavin concentration) or alternatively is present in flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD). Under alkaline pH, irradiation cleaves the ribitol portion of riboflavin, forming a strong oxidizing agent, lumiflavin. Lumiflavin can oxidize other compounds in milk, such as vitamins particularly ascorbate (Fox & McSweeney, 2009).

2.5.1.3. *Current processing methodologies; Pasteurisation*

Pasteurisation is a thermal process widely used in the food and dairy industry to extend shelf life and importantly to minimize health risks from pathogenic microorganisms associated with milk. There are several time-temperature combinations to pasteurise milk, which range from 63°C for 30 minutes (LTLT) or 72°C for 15 seconds (HTST) (Fox & McSweeney, 1998; Ranjith, 2000; Grant *et al.*, 2002; Kessler, 2002). As for UV radiation, heat resistance of microorganisms and

resulting pasteurisation efficiency will be determined by a number of factors. These include, but are not limited, to the following: The type of microorganism; the optimal growth temperatures of the microorganism; the cellular lipid content of the microorganism (i.e. an increase in lipid content increases heat resistance of the organism); the tendency of the microorganisms to form clumps and/or clusters (which will increase heat resistance of the colonies or population); the growth stage of the microorganism (microorganism in the logarithmic growth stage will be more heat resistant); the chemical and biochemical composition of the environment or liquid being treated, the pH of the environment (heat resistance will decline as there is outside the optimum pH range of the microorganisms); and the water activity of the food pasteurised (in general there would be a decrease in heat resistance with a decrease in water activity). During refrigerated storage of pasteurised milk, growth of thermophilic bacteria that survives pasteurisation, as well as microorganisms introduced post pasteurisation can reduce the shelf life of fluid milk. The thermophilic microorganisms include Gram-negative (*Pseudomonas* spp.), Gram-positive (*Paenibacillus amylolyticus*) bacteria as well as spore forming bacteria (Rossitto *et al.*, 2012).

In order to regulate and control the quality of raw milk and to limit the risk of bacterial and possible pathogen contamination, each country enforces regulations defining acceptance criteria for raw milk to be used for further processing. For example, in South Africa the legal specification for the acceptance of raw bulk milk for further processing according to the “Regulations Relating to Milk and Dairy products” (published under Government Notice No. R. 1555 of 21 November 1997) are indicated in Table 2.11 (Anonymous, 1999).

In addition to the regulations relating to acceptance parameters for raw milk, there are also specific regulations relating to the microbiological quality and safety of the final product produced. In regards to pasteurisation the Grade “A” Pasteurised Milk Ordinance (PMO) in the United States governs pasteurised milk quality (Anonymous, 2009). The U.S. Code of Federal Regulations (CFR) (21CFR 131.3) and the PMO for the treatment of milk specify the time and temperature requirements for the reduction of *Coxiella burnetii* and *Mycobacterium tuberculosis* (Enright *et al.*, 1957; Jay 1996). However, both the CFR and the PMO address the possibility for processing

alternatives to heat treatment (21 CFR 1240.61 and CFSAN, 2002). In this regard it is important to note that the PMO states “*that nothing shall be construed as barring any other pasteurisation process which has been recognized by the Food and Drug Administration to be equally efficient and which is approved by the regulatory agency*” (CFSAN, 2002).

Table 2.12: South African Regulations Relating to Milk and Dairy products: Regulations regarding sale of raw milk for further processing

Parameter	Specification
Antibiotics	Absent
Pathogenic organisms, extraneous matter or inflammatory products	Absent
Clot-Boil test	Negative
Standard Plate Count (cfu.mL ⁻¹)	< 200,000
Coliform bacteria (cfu.mL ⁻¹)	< 20
<i>Escherichia coli</i> (cfu.mL ⁻¹) ⁱⁱ	Absent
<i>Escherichia coli</i> (cfu.0.01mL ⁻¹) ⁱⁱⁱ	Absent
Somatic cell count.mL ⁻¹ ^{iv}	< 500,000
Alcohol Test (68% ethanol v/v)	Negative

Published under Government Notice No. R. 1555 of 21 November 1997.

ⁱⁱ Modified Eijkmann Test

ⁱⁱⁱ VRB Mug Agar Method and dry hydrated film method

^{iv} The Standard Method for Counting Somatic Cells in Bovine Milk is set forth in International Dairy Federation (IDF) Bulletin No. 114 of 1979.

The European Union specifies that the processing conditions relating to pasteurised milk requires the milk to be heat treated at a minimum of at least 72°C for 15 seconds according to Regulation (EC) 2074/2005 (Collins *et al.*, 2000). Although the regulation requires 72°C for 15 seconds as the legal minimum the standard dairy practice is to raise this to 72°C for 25 sec as recommended in the Dairy UK Code of Practice on HTST pasteurisation. This increase in temperature is related to the survival of thermophilic *Mycobacterium avis* subsp. *paratuberculosis*, which has been linked to Crohn’s disease. Research conducted by Grant *et al.* (2002) reported that *Mycobacterium avis* subsp. *paratuberculosis* may survive the minimum heat treatment required by EU Regulation (EC) 2074/2005 (Collins *et al.*, 2000; Grant *et al.* 2002).

In South Africa the regulation relating to pasteurised milk, specifies that milk needs to be pasteurised at 63°C for 30 minutes (LTLT) or 72°C for 15 seconds (HTST), and the resulting quality and microbiological control parameters specified following pasteurisation are indicated in Table 2.12 (Anonymous, 1999).

Table 2.13: South African Regulations Relating to Milk and Dairy products: Regulations regarding sale of pasteurised milk.

Parameter	Specification
Antibiotics	Absent
Pathogenic organisms, extraneous matter or inflammatory products	Absent
Clot-Boil test	Negative
Standard Plate Count (cfu.mL ⁻¹)	< 50,000
Coliform bacteria (cfu.mL ⁻¹)	< 10
<i>Escherichia coli</i> (cfu.mL ⁻¹) ⁱⁱ	Absent
<i>Escherichia coli</i> (cfu.0.01mL ⁻¹) ⁱⁱⁱ	Absent
Phosphatase Test ^{iv}	Negative
Alcohol Test (68% ethanol v/v)	Negative

Published under Government Notice No. R. 1555 of 21 November 1997.

ⁱⁱ *Modified Eijkmann Test*

ⁱⁱⁱ *VRB Mug Agar Method and dry hydrated film method*

^{iv} *Aschaffenburg and Mullen phosphate test*

As far as the chemical and biochemical composition is concerned, HTST pasteurisation will affect some of the components in milk, however, these changes will be directly correlated to the severity of heat treatment and time of heating. The denaturation of proteins is mainly restricted to the denaturation of the whey proteins, excluding α -lactalbumin, which is relatively heat stable. Furthermore, no breakdown of peptide linkages would occur, and as a result casein is considered as a thermal-resistant compound. Renner (1986) reported that denatured proteins are more digestible than their naturally occurring form, because the protein's structure has changed, resulting it to be more accessible to digestive enzymes. Beddows & Blake (1982) further confirmed that pasteurisation does not impair the nutritional quality of milk fat, calcium, and phosphorus nor does it affect fat-soluble vitamins (A, D, and E) and water-soluble vitamins (B-vitamins, riboflavin, pantothenic acid, biotin, and niacin). The losses of vitamins caused by pasteurisation, such as thiamin (< 3%),

pyridoxine (0–8%), cobalamin (< 10%) and folic acid (< 10%) are considered lower than those that take place during the normal handling and preparation of foodstuffs at home (Lund, 1982). Most of the vitamin C is lost during handling, pasteurisation, packaging, and oxidation of milk. Renner (1986) further reported that about 70% of the remaining vitamin C and 90% of riboflavin could be destroyed by sunlight exposure during storage. In Table 2.13 some of the major changes in milk constituents are summarized following different levels of heat treatment.

Table 2.14: Comparison between HTST, ESL / extended HTST and UHT pasteurisation and the resulting effect on some of the milk components following heat treatment.

Characteristic	HTST Pasteurisation	ESL / extended HTST	UHT
Heating temp. and time	72°C for 15 seconds	120 – 135 °C for 4 – 1 seconds	135 – 145 °C for 10 – 2 seconds
Heat enzyme inactivation index	Phosphatase negative	Phosphatase negative	Phosphatase negative
Storage conditions	Peroxidase positive	Peroxidase negative	Peroxidase negative
Type of packaging	Refrigerated	Refrigerated	Ambient
Average shelf-life	Clean	Ultraclean / Aseptic	Aseptic
Lactulose (mg.l ⁻¹)	8 – 14 days	30 – 60 days	6 – 12 months
	0	20 – 40	80 - 500
		Gallmann, (2000); Ranjith, (2000)	
Furosine (mg.g ⁻¹) of protein)	0	200	400 – 1200
		Fredsted <i>et al.</i> , (1996)	Gallmann, (2000)
α -Lactalbumin denaturation (%) ^a	~ 5	~ 5	~ 30 – 80
		Fredsted <i>et al.</i> , (1996)	Elliott <i>et al.</i> , (2005)
β -Lactoglobulin denaturation (%) ^b	~ 13	~ 22	~ 60 – 100
	Fredsted <i>et al.</i> , (1996)	Fredsted <i>et al.</i> , (1996)	Elliott <i>et al.</i> , (2005)
			Burton, (1988)
Immunoglobulin denaturation (%)	~ 67	~ 100	~ 100
	Fredsted <i>et al.</i> , (1996)	Fredsted <i>et al.</i> , (1996)	Elliott <i>et al.</i> , (2005)

^a Assuming the concentration of α -Lactalbumin is 1,200 mg.l⁻¹ in the raw milk

^b Assuming the concentration of β -Lactoglobulin is 3,000 mg.l⁻¹ in the raw milk

2.5.1.4. *Novel processing technology; UV radiation of milk*

Novel, non-thermal technologies such as pulsed electric fields, ultraviolet light processing, ionizing radiation, microwave processing, ultrasound processing and batch or continuous high pressure processing are being applied in food processing as a viable alternative to thermal processing (Guerrero-Beltrán and Barbosa-Cánovas, 2004). The emphasis on developing novel non-thermal processing technologies is to improve and retain the quality and nutrition of food products, while still delivering foods that are safe to consume without hazardous microorganisms (pathogens) (Knorr, 1999).

One of the main obstacles to consider when using UV radiation as an alternative processing technology to pasteurisation is the possible introduction of photochemically induced off-flavours and odours. It has been well reported that both UV and visible light are key factors in creating flavour defects and malodours in milk (Bekbölet, 1990; Borle *et al.*, 2001). Past studies also investigated the potential of utilizing UV irradiation for vitamin D enrichment at the specific germicidal wavelength of 254 nm and did not report negative sensory data (Caseiro *et al.*, 1975).

Lipid oxidation, which can lead to oxidative rancidity, is the major cause of defects in milk and dairy products and has been extensively reviewed especially in regard to processing and packaging development that would reduce oxidation. Lipid oxidation is an auto-catalysed free radical chain reaction, which principally involves the oxidation of polyunsaturated fatty acids (PUFA), although saturated fatty acids can also be oxidized. Oxidation ultimately leads to formation of hydroperoxides, which is relatively unstable, and will further breakdown to various other products, such as unsaturated carbonyls, which is mainly responsible for organoleptic defects (Fennema, 1996; Frankel, 1998). Oxidation can be initiated through the action of heavy metals, enzymes and light and in presence of photosensitizer, such as riboflavin (Borle *et al.*, 2001).

The burnt (or sunlight) flavour associated with oxidized milk is typically blamed on lipid oxidation, but protein oxidation has been shown to contribute significantly to the deterioration of milk quality and the production of similar off flavours (Hui, 1993).

Jung *et al.* (1998) postulated that methionine reacts with singlet oxygen produced by riboflavin under lighted conditions to yield dimethyl disulfide, methyl sulfide and other sulfur-containing compounds. By-products of this reaction are ammonia and carbon dioxide that give milk a similar odour to that of lipid oxidized milk (Hui, 1993).

Limited studies have reported the use of UV-C radiation to enhance the microbiological quality and safety of goats and cow's milk (Matak, 2004; Matak *et al.*, 2005; Reinemann *et al.*, 2006; Altic *et al.*, 2007; Donaghy *et al.*, 2009; Choudhary *et al.*, 2011; Rossito *et al.*, 2012). There are few reports on the effect of UV-C radiation on the chemical and biochemical composition of the milk being treated, hence this remains an area of interest for future studies (Matak *et al.*, 2007). The results of the germicidal efficacy on cow and goat's milk studies are summarized in Table 2.4 and are briefly discussed below.

Matak (2004) determined that UV processing was effective as a non-thermal process for the reduction of certain bacterial pathogens in goat's milk. The target pathogen reduction was achieved when the flow rate of the milk through a thin-film turbulent flow UV processing unit (CiderSure 3500 apparatus, FPE Inc., Macedon, NY). Matak (2004) found efficacy of UV-C on inactivation of a naladixic acid resistant *E. coli* ATCC 25922, an *E. coli* O157:H7 surrogate, was highest with skimmed cow milk (2.3 logs) followed by reduced fat milk (1.8 logs) and whole milk (1.4 logs) at different temperatures (between 4 and 20°C) under laminar flow ($Re < 1,371$) at 151 L.h⁻¹ conditions at UV-C dosage of 5.3 – 6.3 mJ.cm⁻² for approximately 1.5 seconds exposure time. Matak (2004) concluded that the processing temperature did not significantly impact the germicidal efficacy of UV-C processing, and factors such as the % Totals Solids had a greater influence on bacterial reductions achieved.

Matak (2004), further reported a greater than 5-log reduction of *Listeria monocytogenes* in inoculated goat's milk exposed to a cumulative UV-C dose of 15.8 ± 1.6 mJ.cm⁻² for a total exposure time of 18 seconds (567 L.h⁻¹ flow-rate) using the same thin-film UV processing unit under turbulent-flow conditions (Matak, 2004; Matak *et al.*, 2005). She concluded that that UV irradiation could be used for the reduction of *Listeria monocytogenes* in goat's milk. During the experimental

protocols followed, Matak (2004) observed off-odours following the UV radiation of the goat's milk and concluded that it could be due to defects catalysed by UV exposure, lipase activity (as a result of turbulent-flow) and agitation during UV treatment or a combination of both. The same thin-film UV processing unit and processing conditions was used to assess the extent of lipid oxidation and hydrolytic rancidity. The thiobarbituric acid reactive substances (TBARS) and acid degree values (ADV) in the milk was analysed after UV exposure at UV-C dose of $15.8 \pm 1.6 \text{ mJ.cm}^{-2}$ for a total exposure time of 18 seconds at 567 L.h^{-1} . The TBARS values increased approximately 2-fold from 0.31 ± 0.09 (0 mJ.cm^{-2} UV dose) to $0.58 \pm 0.11 \text{ mg.l}^{-1}$ (15.6 mJ.cm^{-2} UV dose), while the acid degree value (ADV) increased approximately 4-fold from 0.10 ± 0.05 (0 mJ.cm^{-2} UV dose) to $0.40 \pm 0.09 \text{ mEq.100 g}^{-1}$ (15.6 mJ.cm^{-2} UV dose). However, the increase in the ADV was rather attributed to the possible damage to the milk fat globule membrane and resulting lipase activity, as milk pumped through the UV system without the UV lamps switched on yielded the same results. In general an ADV value of $> 1.0 \text{ mEq.100 g}^{-1}$ in cow's milk would be indicative of rancid off-flavours. No significant changes were found in the fatty acid composition, specifically no changes in oleic acid (C18:1). Solid phase micro-extraction and gas chromatography (SPME-GC) was utilized to quantify the production of volatile compounds. The formation of pentanal, hexanal and heptanal was identified after as little as 1.3 mJ.cm^{-2} UV dose, with peak areas being measured after 7.8 mJ.cm^{-2} and 15.6 mJ.cm^{-2} (Matak, 2004; Matak *et al.*, 2007). Matak (2007) concluded that the chemical analyses results supported the findings from the sensory studies, which could detect the differences in control milk (0 mJ.cm^{-2}) when compared to the UV irradiated milk (15.6 mJ.cm^{-2}).

Employing a thin film turbulent flow UV system (SurePure SP40, SurePure AG, Switzerland), UV-C was also reported to inactivate background flora present in the raw cow milk by 2.29 log of SPC, 2.55 log of psychrotrophs, and 1.67 log of thermotolerant bacteria (Reinemann *et al.*, 2006). The relevance of the study is significant as tests were conducted on a commercial UV-unit with much higher flow rates when compared with some of the other UV systems used in research to date. The results are discussed in more detail in Chapter 4.

Both Altic *et al.* (2007) and Donaghy *et al.* (2009) investigated the germicidal efficacy of UV radiation on the microorganism *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The significance of this research is due to MAP not being inactivated by conventional HTST pasteurisation as also indicated in Table 2.8. Altic *et al.* (2007) achieved between a 0.5 – 1.0 log reduction at a flow-rate of 168 mL.min⁻¹ using a laboratory scale UV apparatus delivering a UV dose of 1000 mJ.mL⁻¹. Donaghy *et al.* (2009) reported a 0.1 – 0.6 log reduction on MAP, using a thin film turbulent flow UV system (SurePure SP2) at a flow rate of 3800 L.h⁻¹ and a UV dosage of 1000 J.L⁻¹.

Choudhary *et al.* (2010) evaluated *E. coli* W1485 inactivation in inoculated milk with a coiled tube UV reactor (SBL325, American Ultraviolet Company, Lebanon, IN, USA) at different flow rates to determine a minimum residence time for a 5-log reduction. The flow rates ranged from 25 to 100 mL.min⁻¹ and *E. coli* W1485 was inactivated > 8.5 log at flow rates up to 75 mL.min⁻¹, corresponding with a minimum residence time of 11.3 seconds.

The decimal reduction time (D-value) of different organisms in full-cream milk were determined by Rossito *et al.* (2012) using the thin film turbulent flow UV equipment (SurePure SP4) and are indicated in Table 2.14. For this determination milk was spiked with the microorganism in question to an approximate final concentration of approximately 10⁷ cfu.mL⁻¹. The dose response was then repeated six times and UV-C dosages applied with the SurePure UV system ranged from 0 – 8800 J.L⁻¹ (Rossito *et al.*, 2012). These organisms tested present the major pathogens associated with foodborne milk outbreaks and spore-forming bacteria associated with the spoilage of HTST milk. In the study they observed less than 3-log reduction on all the organisms at 880 J.L⁻¹ and according the sigmoidal inactivation curve dose response, a plateau was reached at 1760 J.L⁻¹. However, based on the initial concentration of the pathogens inoculated and the D-values reported, the plateau phase could have been as a result of the detection limits of analysis. All the pathogens would exhibit a greater than 5-log reduction based on the D-values given in Table 2.14 after 1760 J.L⁻¹ as per their experimental data. Rossito *et al.* (2012) postulated that this phenomenon was rather as a result of suspended solids that may have negatively impacted on the absorption of UV radiation applied in this instance.

They further concluded that the UV treatment (at 880 and 1760 J.L⁻¹) greatly reduced the growth of psychrotrophic and mesophilic bacteria during a shelf-life study conducted at 4°C and 7°C compared to pasteurised milk (batch pasteurised LHST, 63°C for 30 minutes). Sensory triangle tests conducted on the milk according to the study of Rossito *et al.* (2012) confirmed differences between the UV treated and pasteurised control milk. The main defects associated with the UV treated milk according to panellists' descriptors were 'burnt', 'off', 'strong' and 'stale' taste and aroma. However, they also concluded that most of the panellists could not taste the difference between the treatments and acknowledged that the method used was non-quantitative and do not assess the degree of difference between the samples evaluated. They postulated that the changes in organoleptic profile could be attributed to lipid oxidation, due to oxidative rancidity (increase in acid degree value measured) and an increase in thiobarbituric substances indicative of lipid oxidation as reported by Matak *et al.* (2007). However, the study of Matak *et al.* (2007) focused on goat's milk exclusively and caution should be applied extrapolating results due to the difference in fat profiles between the different milk species (i.e. goat vs. cow's milk). Various authors reported that oxidative stability of milk is proportional to the percentage of unsaturated fatty acids in milk, i.e. the higher the percentage unsaturated fatty acids in milk the inferior the oxidative stability would be (Havemose *et al.*, 2004; Kristensen *et al.*, 2004; Hedegaard *et al.*, 2006). Rossito *et al.* (2012) concluded that before recommending UV technology as an adjunct or alternative to pasteurisation, further studies should be conducted in order to try and limit the 'negative sensory impact' of UV radiation, and that such studies should focus on the dose rate, radiation path, flow-turbulence and UV wavelength as a possible solution (Smith *et al.*, 2002; Engin & Karagul Yuceer, 2012). Some concern is also expressed as to the penetration of UV light into the medium being treated due to the turbidity and solids content of the milk, unlike pulsed UV, which according to them, could be used to adequately control the bacterial content of milk.

Table 2.15: D-values for pathogens and spore formers using a thin film turbulent flow UV system (SurePure SP4) (Rossitto *et al.*, 2012).

Microorganism	ATCC	D-value (J.L ⁻¹)	SE	5-log reduction
<i>Escherichia coli</i> O157:H7	ATCC 43888	334	27.98	1670
<i>Salmonella enterica</i> serovar Senftenberg	ATCC 43845	365	22.91	1825
<i>Yersinia enterocolitica</i>	ATCC 9610	311	30.01	1555
<i>Staphylococcus aureus</i>	ATCC 29213	335	17.68	1675
<i>Campylobacter jejuni</i>	ATCC 33560	354	24.41	1770
<i>Serratia marcescens</i>	ATCC 13880	352	34.92	1760
<i>Aeromonas hydrophila</i>	ATCC 7966	293	24.06	1465
<i>Listeria monocytogenes</i>	ATCC 43256	350	31.69	1750
<i>Bacillus cereus</i>	ATCC 4324	1250	65.66	6250
<i>Bacillus licheniformis</i>	ATCC 14580	294	28.49	1470
<i>Bacillus pumilus</i>	ATCC 72	1250	82.49	6250
<i>Bacillus subtilis</i>	ATCC 6051	770	54.27	3850
<i>Paenibacillus lautus</i>	ATCC 43898	1430	137.49	7150

* UV treatment at 254 nm (from 0 to 8,800); mean D-value at 6 °C of six runs.

2.5.2. Cheddar cheese

More than 1000 varieties of cheese are produced in the world today with a diversity of flavours, textures and forms. Cheese is regarded as a group of fermented milk-based food products, which can be divided into four groups based on the method used to coagulate the milk: (1) rennet coagulated cheeses (representing approximately 75% of the total production globally and, which includes most major international cheese varieties); (2) acid coagulated cheeses (e.g., Cottage cheese and Quark); (3) heat and acid coagulated cheeses (e.g., Ricotta); (4) concentrated or crystallized cheese such as “Brunost or Mysost” produced in Norway (Fox *et al.* 2000).

Approximately one third of the milk produced globally is used for the manufacture of cheese, estimated at more than 20.4 million metric tons in 2011, with the revenue representing approximately 30% of the total dairy product sales revenue, according to the Food and Agriculture Organization of the United Nations (Farkye, 2004). The United States (~23%) and European Union (~44%) accounts for approximately 67% of the total cheese production volume, while South Africa produces about 82 000 metric ton of cheese per year from 800 million litres of milk. About 51% of this

quantity is in the form of Cheddar (31%) and Gouda (20%) and the rest consists of Mozzarella, Feta and Cream cheese (OECD-FAO, 2012).

Many of the cheese production in the world takes place on smaller scale, hence delivering a rich diversity in the range and type of cheeses available (Fox *et al.*, 2004). All cheeses, whether rennet or acid set, can be classified as soft, semisoft (semi-hard), hard, or very hard, depending on their moisture content. The fat in dry matter content (FIDM) is also used for classification purposes, as according to regulatory standards in many countries specific types of cheeses need to conform to specified FIDM values. Although this classification is arbitrary, it helps to systematically group together cheeses that are alike in certain basic features or characteristics (e.g. moisture content), as moisture determines the body, consistency and texture, shelf life and ultimately the character of cheese.

Cheddar-style cheese is one of the most important cheese varieties, which originated around the 12th century in the village of Cheddar, at the foot of the Mendip Hills, in England. It is described as a hard variety, pressed-curd, rennet coagulated cheese, which is produced on large-scale globally.

2.5.2.1. *Cheddar cheese processing*

The production of Cheddar cheese consists of several processing steps as summarized in Figure 2.7. The basic steps followed by Cheddar cheese manufacturing includes the standardization of the raw milk, heat processing (if applicable), fermentation or acidification, coagulation, cutting of the curd, draining of the whey, salting, moulding and maturation.

The milk is usually pasteurised in order to destroy any harmful bacteria and then standardized to the desired casein: fat ratio of 0.67-0.72 :1.0 (Fox *et al.* 2000). Starter cultures (*Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis* and *Streptococcus thermophilus*) and rennet are then added to the warm milk at ~ 30°C) to acidify and coagulate the milk. The coagulum is cut and cooked (scalded) to 37-39°C for ~30 min and held at this temperature for approximately 60 minutes. The heating or

scalding promotes water expulsion from the coagulum particles, which is now called 'curd' and promotes the steady production of lactic acid from the starter culture. At a chosen point, stirring of the curd will stop in order for the curd to settle at the bottom of the cheese vat. The whey is then drained and the curd will mat together to a uniform mass, which is then cut in large slabs of curd. Cheddaring then commences, which involves piling the slabs of curd on top of one and another with regular turning, stacking and restacking. When the pH has dropped from around 6.1 to 5.2 - 5.3, the curd is milled to form small-sized cheese curds, salted, and pressed into blocks or moulds. The blocks are then packaged and ripened for about 3 months to two years.

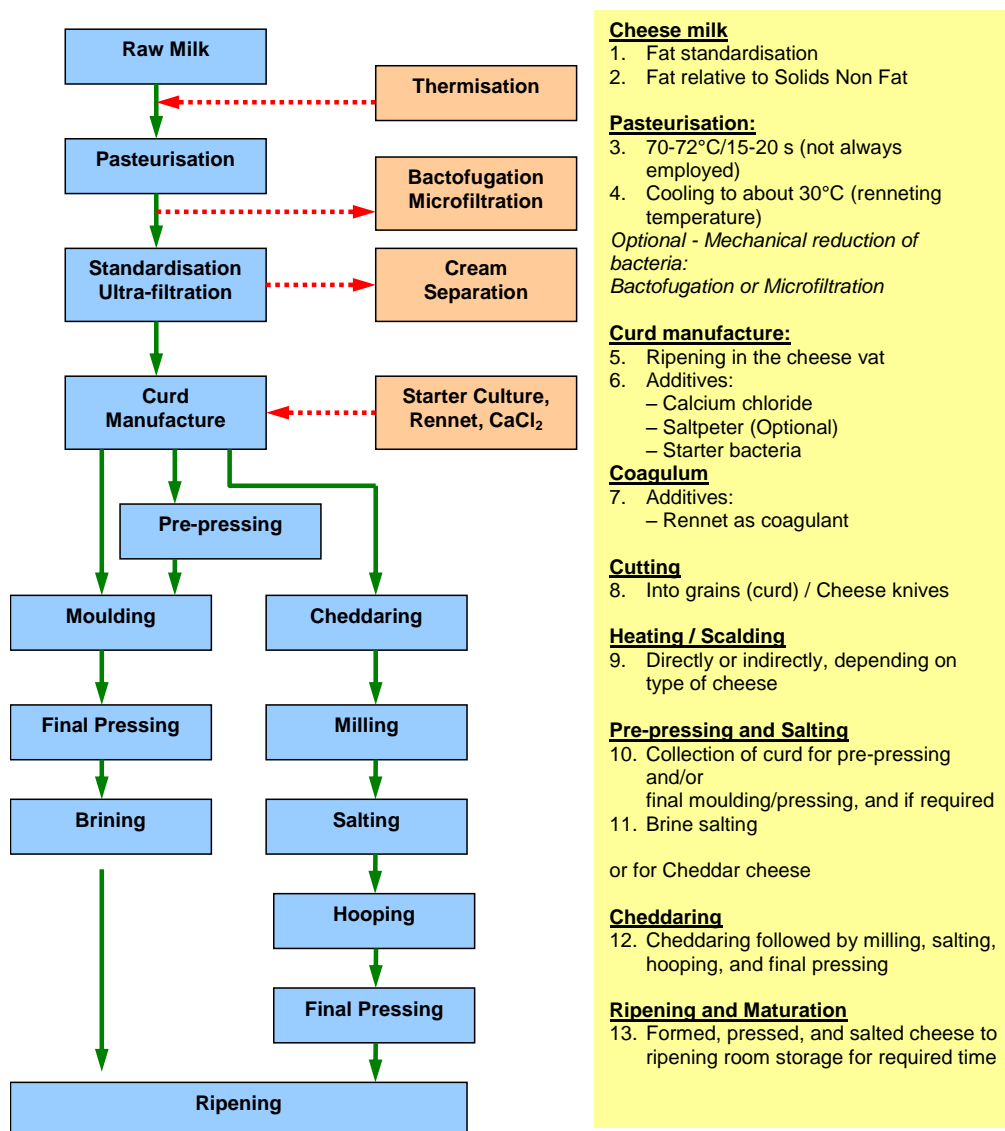


Figure 2.7: Basic manufacturing process for manufacturing of hard and semi-hard cheese varieties, including Cheddar cheese.

2.5.2.2. Cheese ripening

Cheese ripening is a complex biochemical process that can be characterized by three main enzymatic reactions, namely glycolysis, lipolysis and proteolysis as shown in Figure 2.8 (Collins *et al.* 2003; Fox *et al.* 2004; McSweeney, 2004). These reactions are catalysed by rennet, indigenous milk enzymes and starter and non-starter bacterial enzymes, which ultimately will determine the flavour and texture of the cheese. Other factors that would contribute to ripening and the final flavour and the texture of the cheese, include the length of maturation, temperature of maturation, pH development within the cheese and water activity (Andrews, 1983; Fox *et al.* 2004). Proteolysis during cheese ripening is important, as it will impact on the body, texture and flavour development of the matured cheese. It contributes directly to the flavour of cheese through the formation of peptides and free amino acids as well as providing substrates for secondary catabolic changes, i.e. catabolism of aromatic amino acids and reactions of amino acids with other compounds (Fox *et al.* 2000; Fox *et al.* 2004). Lipolysis results in the formation of free fatty acids (FFA), which are constituents of Cheddar cheese flavour and can be precursors of flavour compounds such as methylketones, alcohols and lactones (Smit *et al.* 2000; Fox *et al.* 2000). The fermentation and conversion of lactose to lactic acid (glycolysis) is the primary reaction during cheese manufacture and affects syneresis, retention of calcium, retention and activity of the rennet and inhibits the growth of contaminating bacteria. During ripening lactic acid is also altered, mainly through the action of nonstarter bacteria. In most cheeses there would be a conversion of L-lactate to D-lactate, such that a racemic mixture exists at the end of ripening. Lactate can also be oxidized by lactic acid bacteria in cheese to products such as acetate, ethanol, formate and CO₂ (Fox *et al.*, 2000; McSweeney & Sousa, 2000)

In total, more than 200 flavour compounds have already been identified in Cheddar cheese, highlighting the significance and importance of balancing the flavour components ('the component balance theory') through correct formulation, manufacturing and storage conditions to produce a safe, palatable, pleasant and acceptable final product to the consumer (Mulder, 1952; Maarse & Visscher, 1996).

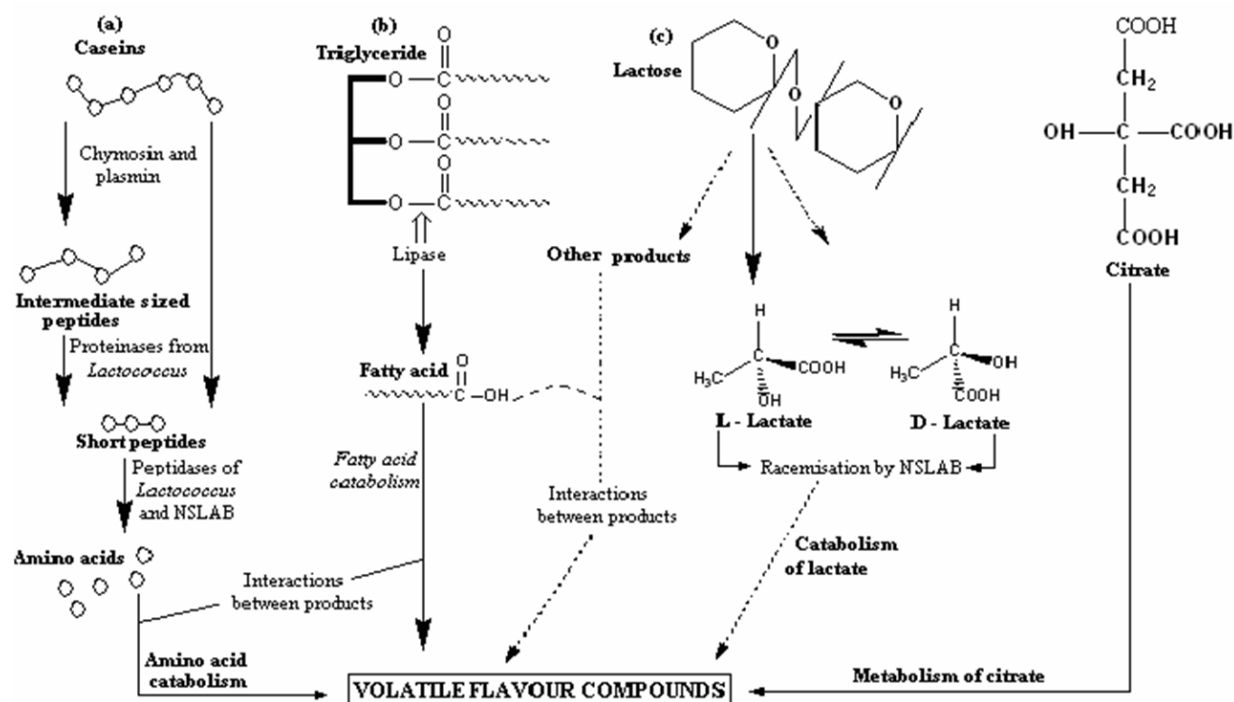


Figure 2.8: Overview of the biochemical pathways during cheese ripening (McSweeney, 2004; Fox *et al.*, 2004).

2.5.2.3. Heat effect on milk and cheese quality

As discussed in section 2.5.1.3. pasteurisation of milk results in a series of physiochemical changes in the milk constituents, depending on the time and temperature of the heat treatment applied. The most significant change to consider with the production of cheese is the denaturation of whey proteins at temperatures $> 60^{\circ}\text{C}$, and interaction of the denatured whey proteins and the casein micelles and the transfer of soluble minerals to the colloidal state. Casein micelles are very stable at high temperatures although changes in zeta potential, size, hydration of micelles, as well as some association-dissociation reactions do occur at severe heating temperatures (Fox & McSweeney, 1998; Fox *et al.*, 2000). Whey proteins are denatured by the unfolding of their polypeptides when heated above 65°C , subsequently exposing the side chain groups originally protected within the native globular structure. Through thiol-disulphide interchange reactions, hydrophobic interactions and ionic linkages the denatured whey proteins will unfold and interact with casein micelles or simply aggregate with themselves (Vasbinder *et al.* 2003).

The interaction of denatured whey proteins with casein is largely between β -lactoglobulin and κ -casein and involves disulphide and hydrophobic interactions (Sandra & Dalgleish 2007). The interaction of whey proteins with the casein micelles can have both positive and negative implications in cheese manufacture, and as a result need to be 'balanced' carefully during cheese manufacture. Denatured whey proteins associated with casein micelles incorporated in the cheese curd, will result in an increased yield from a given quantity of milk. However, the whey protein casein micelles interaction can also impede rennet coagulation, resulting in extended rennet coagulation times (RCT) and weak curd structures (Singh & Waungana, 2001). Weaker curd structure is caused by the disruption of the continuity of the gel network due to the denatured whey proteins sterically hindering the close approach and contact between casein micelles. The resulting coagulum will take longer to form and a weaker, less closed/dense gel-network will form due to reduced cross-linking between casein micelles (Vasbinder *et al.* 2003). The rate of coagulum formation and strength of the gel will further be reduced by factors such as the formation of heat-induced colloidal calcium phosphate (CCP) (Schmidt & Poll 1986). Limited studies have explored the difference in ripening profiles when comparing cheese manufactured from heat treated milk with cheese manufactured from raw milk. As far as proteolysis is concerned, Benfeldt *et al.* (1997) did report that cheese manufactured from heated milk showed a decreased rate of proteolysis of caseins, hence resulting in slower formation of small peptides and free amino acids. Furthermore Benfeldt *et al.* (1997) concluded that as the intensity of heat treatment increases, the protein matrices of cheese become 'coarser' and 'less homogeneous' in appearance, as measured by Scanning Electron Microscopy, and contain numerous small holes or cracks. Singh & Waungana (2001) also concluded that the body, texture and flavour profiles of cheese manufactured from heated milk would be different to cheese manufactured from raw milk.

2.5.2.4. *UV light radiation of milk for the manufacturing of Cheddar cheese*

The use of alternative processing technologies, as alternatives to thermal treatment of various liquids including cow and goat's milk, have been the subject of investigation of various studies as summarized in Table 2.4 in paragraph 2.2. However, the

influence of UV radiation as an alternative to pasteurisation for milk used for the production of secondary dairy products, such as Cheddar cheese, has not been researched.

Lau *et al.* (1991) did compare Cheddar cheese produced from raw cow's milk with pasteurised milk during 6 months of ripening. In the study they found that the Cheddar cheese from pasteurised milk, did yield statistically significant differences when compared to the raw milk Cheddar cheese. Firstly the rate of proteolysis seemed to be lower in the pasteurised Cheddar when compared to the raw milk Cheddar as the 12% TCA-soluble nitrogen components recorded in the pasteurised Cheddar were lower during ripening. Furthermore the breakdown of β -casein and resulting peptides formed was also slower in the pasteurised Cheddar. The concentration of water-soluble nitrogen (WSN) was similar in both variants produced. Lau *et al.* (1991) concluded that the changes could be attributed to the denatured whey protein-casein interactions as a result of pasteurisation, which rendered the caseins less accessible to proteases and subsequent proteolysis. In closing they stated that these differences could affect the flavour development.

Yu & Ngadi (2006) investigated the potential application benefits when processing milk for cheese manufacture using alternative technologies, such as pulsed electric field (PEF). Yu & Ngadi (2006) found that PEF treated milk showed better rennetability when compared to thermally pasteurised milk. The curd firmness (CF), RCT and proteolysis results confirmed that the PEF treated milk could give similar flavour in cheddar cheese as raw milk or even superior to that of pasteurised milk. They concluded that PEF treatment could successfully be used as an adjunct to pasteurisation or could replace pasteurisation with a negligible impact on final product quality and flavour.

The investigation of the effect of UV radiation on various dairy products remains of interest, especially in lieu of the various advances in processing technologies and our better understanding of the product composition and factors that would influence the final product quality. With the advances in processing technology and equipment design, some of the negative effects associated with alternative processing technologies could be negated to some extent by manipulating or changing

conventional processing parameters and methodologies. Furthermore, concentrated products like Cheddar cheese, would be model systems to study the effects alternative processing technologies, such as UV radiation, as possible ‘defects’ would possibly be amplified with the concentration of nutrient components such as fat, proteins and carbohydrates.

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2.7. Connecting text

The development of the SurePure Turbulator™ (ex-PureUV) is discussed in Chapter 3. Specific reference is made to the hydrodynamic model to elucidate the critical design parameters associated with the UV dose responses on various microorganisms within bovine milk. Measurable outputs were the registration of a patent in regards to the SurePure Turbulator™, and the construction of commercially available UV treatment systems incorporating the SurePure Turbulator™ technology.

Chapter 3

A novel thin-film, turbulent-flow ultraviolet (UV) system for milk treatment

NOTE: Due to the patent registration and intellectual Property Rights of SurePure AG, Switzerland the information in Chapter 3 should be regarded as confidential, unless in public domain as per the peer reviewed article of Simmons *et al.* (2012) and Patent registration document Patent number US 6,916,452 B1 (Annexures A & B).

3.1. Introduction

Incident UV radiation is attenuated by $> 90\%$ over a path length of less than a millimetre in turbid liquids such as milk and whey (Koutchma, 2009). As a result the design of the reactors or turbulators should ensure that the required UV radiation dose is received before the radiation is attenuated. Various design parameters have been used in UV-C reactors or turbulators to employ ‘thin film’ or ‘surface refreshment’ design features either as a stand-alone design feature or in combination, as in the case of the SurePure (SP) TurbulatorTM

3.2. Critical design features of a novel thin-film, turbulent-flow UV system for milk processing

The design features incorporated in the SP thin-film, turbulent-flow UV system specifically promotes 4 key points in order to achieve the desired germicidal efficiency in the liquid being treated, in this instance milk. It is important that these critical operating parameters must be adhered to when applying the SP technology using the current turbulator design specifications:

1. Maintain the minimum flow-rate of 3800 litres per hour and minimum flow velocity of $1.5 - 3.0 \text{ m.s}^{-1}$.
2. The UV-C intensity of all the lamps should be $> 300 \text{ } \mu\text{W/cm}^2$ when measured at 1 meter ($> 25 \text{ UVC Watts}$).
3. UV-C Dosage applied can vary depending on the quality of the milk and the desired germicidal efficacy needed. Typical dosage values will range between $250 - 3000 \text{ J.L}^{-1}$.
4. Turbulent flow should be maintained through the SP system and the Reynolds value should be > 4500).

3.2.1. The SurePure TurbulatorTM

The commercial SurePure TurbulatorTM system, designed and manufactured by SurePure AG, Switzerland, was used in this study. The system consists of 40 single “turbulators” (SP-40) connected in series. A single UV turbulator applies a novel swirl tube design promoting ‘thin film’ or ‘surface refreshment’ and consists of a stainless steel inlet and outlet chamber, with a corrugated spiral tube connecting the inlet and outlet chambers. The tangential inlet of the reactor creates a high velocity and turbulence in the inlet chamber and brings the whole volume of the liquid into the contact with the UV photons. Inside the spiral tube a low-pressure mercury UV-C lamp, protected by a quartz sleeve, is housed. The liquid flows in the gap between the corrugated spiral tube and the quartz sleeve at a minimum flow rate (F_r) of 4000 L.h^{-1} (1.11 l.s^{-1}) with a Reynolds value (Re) in excess of 6000, indicating turbulent flow pattern as confirmed by Simmons *et al.* (2012) and the equipment manufacturer. The UV light intensity on the surface of the quartz sleeve was 17.7 mW.cm^{-2} as measured by the lamp manufacturer, using calibrated radiometer. Figure 3.1 shows a cross-sectional view of a typical SurePure TurbulatorTM as used in this research project.

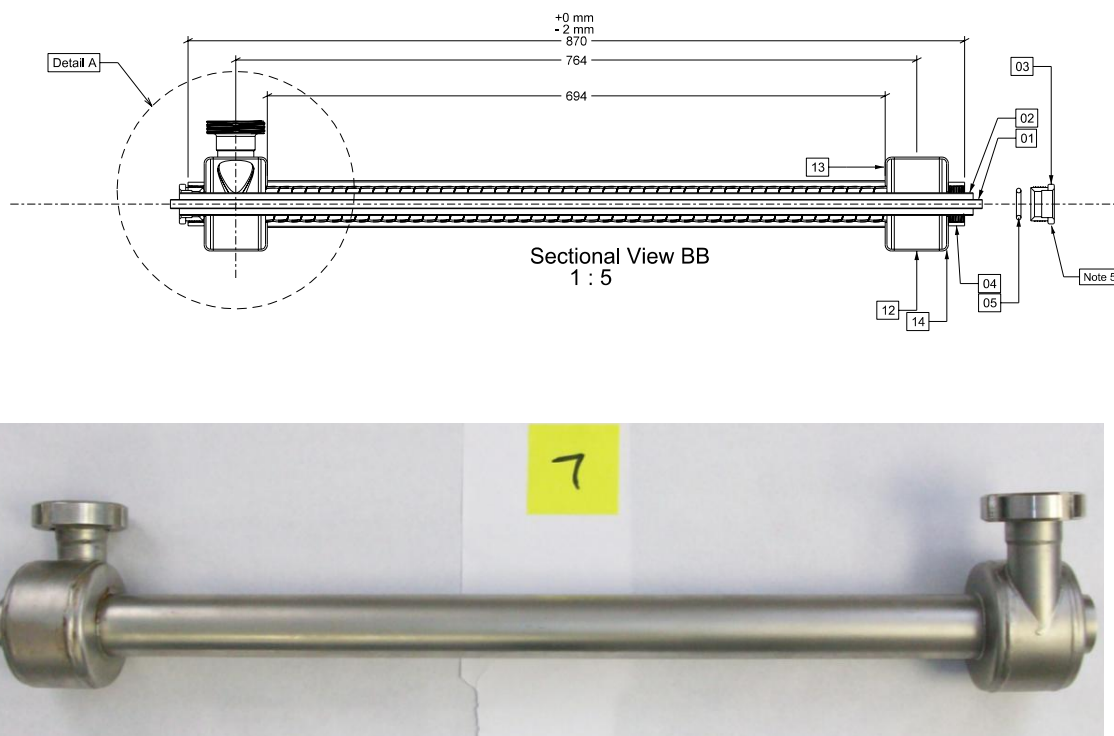


Figure 3.1: Cross sectional view and outside view of the SurePure Turbulator™.

The SurePure Turbulator™ carries a patent registration (Patent number: US 6,916,452 B1) as per Annexure A. The US 6,916,452 B1 patent registration also forms the basis of various other patents registered to manufacturers that uses the turbulator technology under license of SurePure AG, Switzerland. These patent registrations include a novel calves milk pasteuriser, UV Pure™, trademarked, manufactured and distributed by GEA Farm Technologies, Illinois, USA (Patent number: US 8,448,569 B2) as per Annexure B.

3.2.2. Source of UV radiation

The SurePure UV system uses a non-ozone generating, 100 W low-pressure mercury lamps (Model 2364, First Light Technologies, Poultney, USA) as a source of UV-C, which is powered by 230 V ballasts (Workhorse 5, WH5, First Light Technologies, Poultney, USA). The lamps generate 29 UV-C Watts ($280 \mu\text{W}\cdot\text{cm}^{-2}$ at 1 meter) with a rated lamp life of approximately 9,000 hours (% UV-C output after 9000 hours > 60%). The lamps are housed within quartz sleeves and do not come into direct contact with the liquid being treated.

3.2.3. Flow-dynamics

The hydrodynamic performance of the SurePure TurbulatorTM is well described in a study of Simmons *et al.* (2012). In the study Particle Image Velocimetry (PIV) and Positron Emission Particle Tracking (PEPT) were employed to qualify critical design elements and operational efficiency of the SurePure TurbulatorTM in regards to the UV radiation and subsequent germicidal efficacy (microbial kill-rate) when treating turbid liquids, such as milk. Simmons *et al.* (2012) established a microbial reduction model based on the PEPT (which can be applied to turbid liquids) data generated, which takes into account the fractional time density and functional distance of the liquid being treated from the source of UV. They concluded that the geometry of the SurePure TurbulatorTM and the tangial inlet promoted a 'swirling motion' (turbulent flow patterns) and radial mixing thus also increasing 'refreshment' of the liquid to be in more frequent contact with the UV-source surface. The design elements of the SurePure TurbulatorTM increased the microbial kill-rate by approximately 100% when compared to a plug-flow (laminar flow) model, thus the germicidal efficacy was shown to have strong correlation to flow-rate and the tangial inlet design of the SurePure TurbulatorTM.

3.2.3. Geometric configuration

The geometric configuration of the SurePure TurbulatorsTM will ultimately determine the UV-C dosage delivered to the liquid being treated. The arrangement of the turbulators in series in a continuous system will increase the exposure time and the resulting UV-C dosage delivered to the liquid being treated at a constant flow-rate. The nomenclature of the systems depicts the number of turbulators arranged in series, i.e. SP 4 has 4 turbulators, SP10 has 10 turbulators and a SP40 has 40 turbulators in series. The arrangement of the turbulators can therefore be adjusted for the liquid being treated according to the dose model needed to achieve the desired kill-rate towards the target microorganism. A positive displacement pump feeds the system to ensure that the flow-rate through the SP system remains constant, regardless of the backpressure created. A typical flow diagram of the SurePure technology in a dairy

plant is illustrated in Figure 3.2 and the arrangement of the turbulators in the SurePure SP-40 commercial unit is illustrated in Figure 3.3 and 3.4:

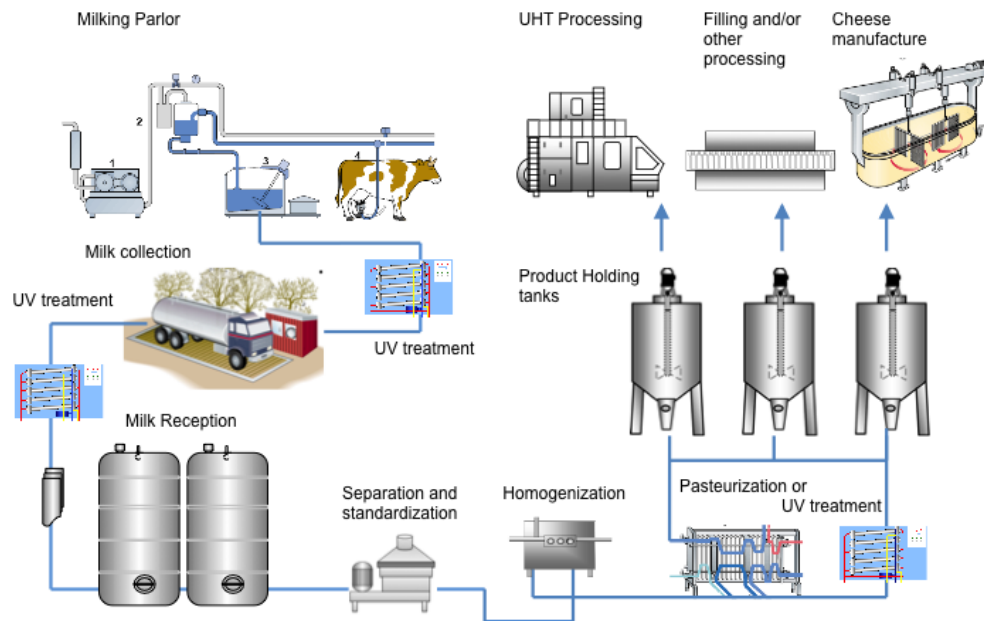


Figure 3.2: Flow diagram indicating the possible entry points within a normal dairy processing line for the SurePure commercial systems.



Figure 3.3: Commercial SurePure SP-40 system's geometric configuration with 40 turbulators in series delivering 1 kJ.L^{-1} of UVC dosage at a flow-rate of 4000 L.h^{-1} .



Figure 3.4: Outside view of the SurePure SP-40 commercial system with 40 turbulators in series delivering 1 kJ.L^{-1} of UVC dosage at a flow-rate of 4000 L.h^{-1} .

3.2.4. UV-C dose calculation for the SurePure UV-system

The UV-C dose calculation and delivery remains one of the most important parameters determining UV dose response against targeted microorganisms, ultimately determining the efficacy of the UV equipment used for the disinfection of liquids.

The limitations of the ‘conventional UV-C Dosage measurement expressing UV dose per area is obvious when treating liquids (e.g. mJ.cm^{-2} , Matak *et al.*, 2005). This characterization is difficult to apply for the treatment of liquids, which may have very different depths of treatment liquid. Reinemann *et al.* (2006) proposed an alternate formula to characterize the UV-dose, calculating the dosage per volume of liquid being treated for single-pass continuous systems, which is described in Chapter 4 (Reinemann *et al.*, 2006 and Keyser *et al.*, 2008). The comparative UV-doses and other critical processing parameter calculations relating to the SP turbulator are shown in detail in Annexure C (UV-dose per area measurement for SP UV system, i.e. the ‘conventional’ calculation method) and Annexure D (UV-dose per volume

measurement for SP UV system, i.e. the ‘Reinemann’ calculation method). In summary, for 1 SP turbulator, the following parameters as shown in Table 3.1 would be applicable for the treatment of full cream milk at a flow rate of 4000 L.h⁻¹:

Table 3.1: Summary of the UV-C dose and processing parameters associated with the treatment of full cream milk at 4000 litres per hour through one SurePure turbulator.

Parameters – 1 X SP turbulator		
<i>Full cream milk: Density (ρ) milk @ 20°C, 1.030kg.l⁻¹ and Dynamic Viscosity (μ), 3.2 centipoise</i>	Value	Unit
Single lamp output (UV-C Watts)	25.5	Watts
Flow-rate (Q) (litres per hour)	4000	L.h ⁻¹
Velocity of flow (V_a) (metres per second)	1.415	m.s ⁻¹
Retention Time (t) (seconds)	0.608	s
Effective UV-C dosage per volume (D_{uv}) (Joules per liter)	23.00	J.L ⁻¹
Effective UV-C dosage per area (D_a) (mJ.cm ⁻²)	23.07	mJ.cm ⁻²
Reynolds Value minimum estimate (R_e)	> 6000	~

3.3. UV Dose response using the SurePure UV-system

Measurement of the UV-dose response against targeted microorganisms in milk was the basis for evaluating design parameters and efficacy of the SurePure TurbulatorTM during research and development. Milk was identified as the liquid of choice to evaluate as it presented a ‘worst case scenario’ to evaluate efficacy of the SP system design. Milk is a complex liquid due to its chemical and biochemical composition and also is the perfect substrate for growth of a diverse microbiological population. Furthermore milk has limited UV penetration due to UV-light attenuation in milk, with the absorption coefficient of full cream milk being < 300 cm⁻¹. The UV dose response measurements and analytical results against targeted microorganisms, was used for mainly three reasons during the initial research and development phase: Firstly, to evaluate the UV efficacy within the ‘standard’ or ‘current’ system design, secondly, to evaluate the UV efficacy when any system design change or processing parameters were implemented and thirdly, once the optimum design parameters were established, to compare the UV efficacy of different microorganisms within the respective liquid being treated. For obvious reasons the microbial efficacy, which is

the primary reason for application, was first evaluated and once desired germicidal efficacy was achieved, further analysis and evaluation were conducted on the impact of UV-C on the chemical and biochemical compounds as well as organoleptic properties in the milk.

3.3.1. Genesis – The first dose response testing; ARC Dairy Laboratory, Elsenburg (Stellenbosch)

The first evaluation of the UV ‘reactor’ design (Hydrazone Pty. Ltd., Tokai) was conducted at the ARC Dairy Laboratory at Elsenburg, in 1998 -1999. The main application identified was the possible use of UV-C within milking parlours to reduce the bulk milk microbial count after milking, with the system being placed between the milking receiver jars and the bulk milk cooling tank at dairy-farms. The idea was that the reduction of microorganisms in the milk on-farm would result in better payment terms for farmers delivering their milk to co-operatives and secondary producers as the payment model to farmers incorporated penalties if the total bacterial and somatic cell count exceeded certain levels. Farmers were also rewarded for delivering A-grade milk, i.e. milk containing less than 50,000 cfu.mL⁻¹ total plate count (TPC). This model proposed numerous challenges as the milk-flow rate between the receiver jars and the bulk milk-cooling tank is erratic and intermittent, hence impairing optimal flow through the UV reactor at the time, also causing burning of the milk in the reactors in periods of no-flow as the flow is stop-start during milking. Furthermore, with not having a continuous flow-stream through the reactor, the lamps in the UV reactor overheated causing UV-C ‘fall-off’ or a drop in UV-C output at temperatures > 80°C, due to non-optimal lamp operating temperatures. Furthermore, although the flow was being regulated between 2800 – 3000 L.h⁻¹, the first system design did not have a corrugated tube as per the system design indicated in Figure 3.1, and only consisted of a pipe without corrugation (i.e. regular milk/stainless steel pipe). As would be expected the system did not deliver optimal results as far as the 2 main parameters were concerned, delivering less than a 50% reduction (less than 1-log) on TPC and coliforms tested after 1 pass through the reactor. Following the same results on different tests conducted, it was concluded that the milk was not exposed sufficiently to the source of the UV-C, and therefore a thin-film, turbulent

flow model needed to be established. The analogy that was used was to create a ‘gun barrel’ effect, thus creating the same ‘swirl-flow’ motion, as would be the case with propelling a projectile through the barrel of a gun. The idea was refined and it was then proposed to replace the non-corrugated tube with a corrugated tube through which the milk would then flow between the corrugated tube and the quartz sleeve housing the UV-C lamp. The corrugated tube was a similar principle and design that is used in tubular heat exchangers to promote heat transfer to the product during pasteurisation and/or sterilization. Further advantages hypothesized were that this turbulent/swirl-flow would also create high and low-pressure areas within the liquid being treated, hence exposing the liquid to the light and breaking-up any bacterial ‘clumping’ that might occur, allowing for more effective UV-C transfer to individual cells, limiting ‘shielding’ of microorganisms to the source of UV-C.

3.3.2. The first UV-reactor prototype testing on a dairy farm; Vyevelei (Malmesbury)

The first reactor design including the corrugated tube was tested at Elsenburg Dairy laboratory early in 2000 and it was found that this new design vastly improved germicidal efficacy when treating milk. The tests results conducted at full cream raw milk from the Elsenburg Dairy herd at 35°C confirmed a > 90% reduction on both TPC and coliforms (1-log reduction on TBC and between a 1 – 1.35 log reduction on coliforms) after one pass through the reactor. The prototype was then tested within a production environment at one of the biggest dairy farms in South Africa, Vyevelei (Malmesbury, Western Cape) that at that stage delivered milk to Parmalat South Africa. Vyevelei produces between 60000 – 70000 litres per milk per day, with a herd size of approximately 2800 dairy cows. Four UV-units were installed, two each in series and four units in total in parallel, mainly to accommodate the flow rate of between ~ 6000 L.h⁻¹ between the milk receiver jars and the bulk milk cooling tanks. The installation configuration therefore allowed UV-C exposure of two reactors to a body of milk, at an approximate flow-rate of between 2800 – 3000 L.h⁻¹ of a body of milk at any given time. Milk was sampled before and after the reactor in specially designed sample cock’s that would allow simultaneous sampling as milk flowed through the system, hence giving an indication of the germicidal efficacy. This method of sampling also did not present an ideal model, as could be seen from the

variation in colony forming units before UV treatment, but did indicate a trend as per the initial results at Elsenburg Dairy laboratory. The reason being that there was no homogenous body of milk that could be sampled before and after treatment as this was done in production, and the method could therefore have incorporated a certain margin of error. Nevertheless, good results were achieved on TBC, coliforms and psychrotrophic bacteria as summarized in Table 3.2. The average kill rate was the highest for coliforms (approximately 2-log reduction), followed by psychrotrophs (approximately 1.5 log reduction) and TPC (approximately 1.0 log reduction).

Table 3.2: Average log reduction of Total Bacteria Count, coliforms and psychrotrophic bacteria in full cream raw milk following UV treatment ($\sim 50 \text{ J.L}^{-1}$ at $2\,800 - 3\,000 \text{ L.h}^{-1}$, milk at $\sim 35^\circ\text{C}$) at a commercial dairy farm in Malmesbury ($n = 140$).

Approx. 50 J.L^{-1} UVC exposure ($n = 140$)	Total Bacterial Count		Coliforms		Psychrotrophs	
Before vs. After	cfu.mL ⁻¹	STDEV	cfu.mL ⁻¹	STDEV	cfu.mL ⁻¹	STDEV
Before (cfu.mL ⁻¹)	215900	96917	2488	970	102644	102644
After (cfu.mL ⁻¹)	39075	67984	81	196	21391	53999
Before (log cfu.mL ⁻¹)	5,244	0,357	3,322	0,321	5,191	0,442
After (log cfu.mL ⁻¹)	4,113	0,682	1,395	0,621	3,723	0,668
Average reduction (log cfu.mL ⁻¹)*	1,131 (2.477)	0,772	1,928 (3.477)	0,754	1,468	0,848 (2.500)

* The highest log-reduction achieved are indicated in brackets.

3.3.3. The first external validation

In order to get independent validation full cream milk was tested at the University of the Orange Free State (UOFS) using the same UV-reactor design used in the experiments at Elsenburg Dairy laboratory and Vyevelei dairy farm. The results of the UOFS correlated well with the results achieved at Elsenburg Dairy laboratory, and is summarized in Table 3.3. In addition to the microbiological testing conducted, some basic tests were included on some of the macronutrient components (Viljoen & Lourens, 2000). No differences were found within all of the components tested and the report concluded that in contrast to previous findings relating to the application of UV as a germicidal agent in milk, “...the patented UV unit in milk, clearly indicated on a significant reduction in microbial loads without affecting the chemical composition.” However caution was expressed in the report, that although a reduction

of > 90% was achieved on the microorganisms being tested, it does not necessarily render the milk safe and that more work should be done in terms of challenge testing of the UV unit. Furthermore it was hypothesized that the advantages of the UV treatment of milk could benefit farmers financially by obtaining better milk grading for payment purposes and that the UV treatment of milk could enhance the shelf-life of milk as well as deliver a final product with better microbial quality (especially for milk processors using the milk for cheese manufacture).

Table 3.3: Results of testing of the UV-system at the University of the Orange Free State. Full cream raw milk was used and exposed to $\sim 50 \text{ J.L}^{-1}$ using the Hydrazone UV reactor.

Parameter	Effect
Aerobic bacterial counts cfu.mL^{-1}	90.1% reduction ($\sim 1 \text{ log}$)
Coliforms cfu.mL^{-1}	93.0% reduction ($\sim 1.5 \text{ log}$)
Yeasts cfu.mL^{-1}	71.6% reduction
Mould cfu.mL^{-1}	92.0% reduction
Pathogens cfu.mL^{-1}	100% reduction
Somatic cell count	28.2% reduction
Fat ¹	No difference
Protein	No difference
Total Solids	No difference
Acidity	No difference
Casein	No difference
Minerals	No difference
Holding ability of milk	4.7 log increase ²

¹ No visible signs of fat oxidation or increase in free fatty acids (fat hydrolysis)

² 4.7 log increase = maximum margin considered by the IDF and SA law to be fit for human consumption

3.3.4. Further system development and the relation of an increased dose response to germicidal efficacy

An extensive review of the germicidal efficacy and performance of the SurePure UV system by Reinemann *et al.* (2006) is described in Chapter 4. However, to illustrate the effect of dose response with relation to: (1) change in critical operating parameters, such as a change in flow rate and (2) germicidal efficacy with an increased accumulative UV-C dose to be delivered to the milk, testing was done in conjunction with the University of the Western Cape to validate the net effect on

germicidal efficacy. In this regard it was important to establish optimum processing parameters and correct UV-C dosage before commencing any further research on a commercial scale as described in Chapters 5 and 6.

Tests were performed using a pilot-scale UV system (SP-4) to treat full cream raw milk at 4°C, and are therefore comparable to the treatment regime followed by Reinemann *et al.* (2006). The tests were conducted at the University of the Western Cape, Biotechnology Department, Cape Town. Different flow-rates, between 2 500 and 4 750 L.h⁻¹, were tested on the SP-4 system. Full cream raw milk and milk with a 2% inoculum was used to compare the efficacy and UV-C dose response. The inoculum was prepared by incubating a predetermined volume of milk to be used for the inoculum at 35°C for 24 hours, before addition to the full-cream raw milk. After inoculation, the milk was kept at 4°C for 2 hours under gentle agitation before UV radiation was applied. The UV-C dosage levels applied ranged from 0 – 2 kJ.L⁻¹ and the milk samples taken before and after UV-C exposure were kept on ice and analysed within 2 hours of being sampled.

The results in Figure 3.5 clearly illustrate the influence of flow-rate in germicidal efficacy when applying UV-C in milk using the SP turbulator. Full cream raw milk of average quality was used, with an aerobic plate count (APC) of less than 150000 cfu.mL⁻¹. The germicidal efficacy in at 3500 – 4500 L.h⁻¹ followed linear microbial inactivation trends with excellent correlation ($R^2 > 0.97$). However at the lowest flow-rate of 2500 L.h⁻¹ the correlation was not as good as at the other flow-rates ($R^2 = 0.85$). At flow-rates of 2500 and 3500 L.h⁻¹, the germicidal efficacy was also less than at the higher flow-rates, possibly due to laminar- or transient-flow patterns as supposed to turbulent-flow through the SP turbulator. At 2500 L.h⁻¹ a 1-log reduction at 2.1 kJ.L⁻¹ UV-C exposure was achieved with a 0.7-log reduction at 1 approximately 1kJ.L⁻¹ at 3 500 L.h⁻¹. In contrast, at flow-rates > 4000 L.h⁻¹ a > 2-log reduction was achieved on all instances at a UV-C dosage of > 1.0 kJ.L⁻¹, with the highest germicidal efficacy of 3.4-log being achieved at 1.1 kJ.L⁻¹ at 4500 L.h⁻¹. The results achieved correlated well with the findings of Reinemann *et al.* (2006) as described in Chapter 4, indicating that flow-rates using the SP turbulator should be ≥ 4000 L.h⁻¹ in order to achieve maximum germicidal efficacy on APC's in raw full-cream milk.

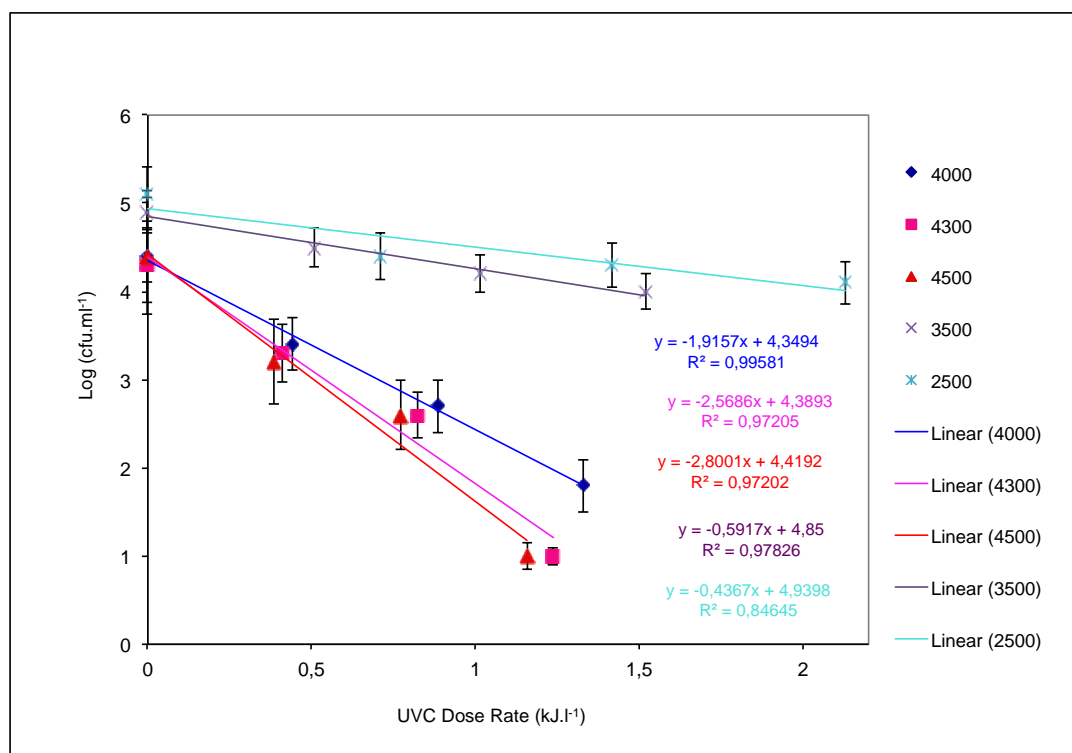


Figure 3.5: Log 10 reduction of APC in fresh (non-inoculated) full cream milk ($n = 3$), after using different UVC dosage (kJ.L^{-1}) levels at 5 different flow-rates (L.h^{-1}).

In Figure 3.6, the results of the germicidal efficacy in the 2% inoculated full cream raw milk is shown. In all instances the initial APC was $> 5.8 \text{ million cfu.mL}^{-1}$ (or $>$ than 6.8-log APC). As per the results achieved from the previous analysis, summarized in Figure 3.5, the flow-rates tested was $>$ than 4000 L.h^{-1} in all instances. Interestingly, the microbial inactivation response did not follow linear trends, as was the case with the non-inoculated milk. The microbial inactivation rather followed a polynomial (2^{nd} order) response with an excellent correlation ($R^2 > 0.94$) in all instances. However, as per the non-inoculated milk, the best results in regards to microbial inactivation were obtained at the highest flow-rate of 4750 L.h^{-1} , estimated at a 5-log reduction at a UV-C dosage approximately 1.5 kJ.L^{-1} . Figure 3.6 also clearly illustrates a definite reduction in microbial inactivation after 1 kJ.L^{-1} of exposure. This observation could be attributed to a sigmoidal inactivation curve as described in section 2.3.4, and the survival of some microorganisms reaching resulting in a final plateau (or tailing) phase during UV treatment. This phenomenon would also greatly depend on the composition of the APC, as well as the possible

population shift occurring during UV-C treatment. Furthermore it is also important to keep in mind that the stage of growth cycle of the organism is also important as UV radiation would be more effective when applied during the lag phase of growth, rather than during the logarithmic growth phase of the organisms, which would also be the case of the inoculated milk (Snowball & Hornsey, 1988). Regardless of the inactivation kinetics observed during the UV-C treatment of both the inoculated and non-inoculated milk, it is noteworthy that in both treatment regimes the final APC counts were between 1 – 2 log APC cfu.mL⁻¹ (or between 10 – 100 cfu.mL⁻¹ APC), which represents a significant reduction in APC in the treated milks.

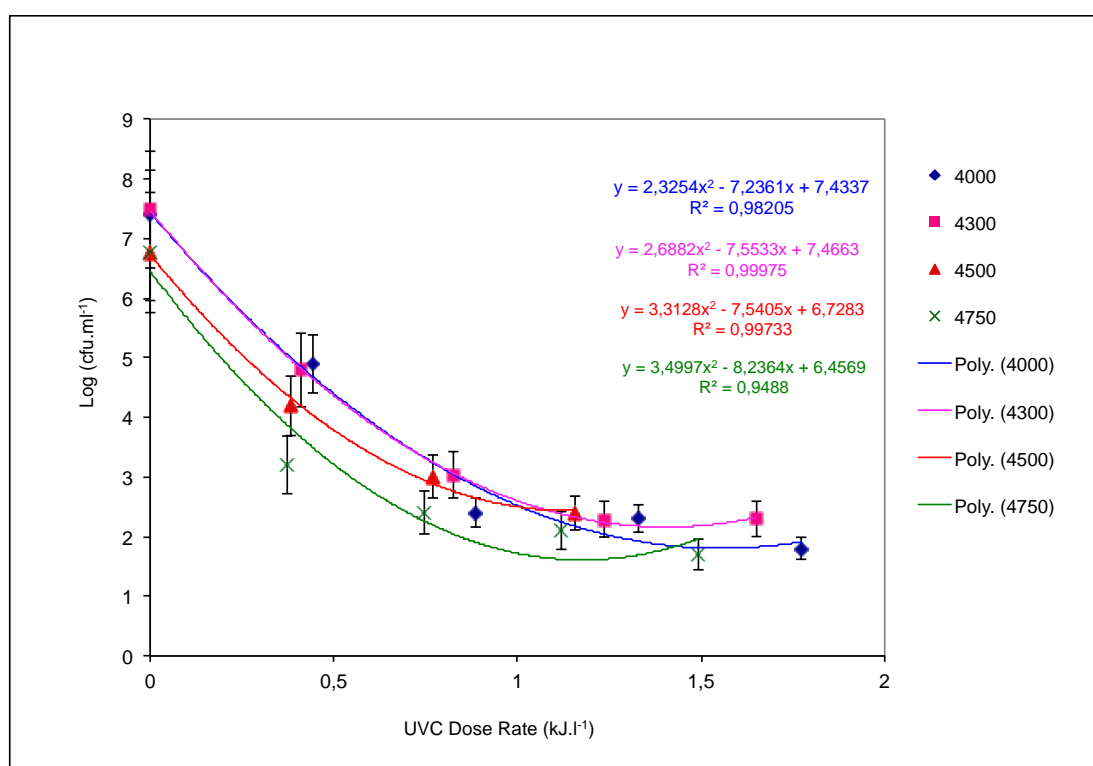


Figure 3.6: Log 10 reduction of APC in inoculated full cream milk (n = 3), after using different UVC dosage (kJ.L⁻¹) levels at four different flow-rates (L.h⁻¹).

In conclusion, a UV-C treatment regime using the SP turbulator at a flow-rate of > 4000 L.h⁻¹ at a UV-C dosage of ~ 1.kJ.L⁻¹, should provide significant advantages when used on a commercial scale as far as the overall improvement of the microbiological quality of the milk is concerned. A UV-C dosage level of 1 kJ.L⁻¹ is considered as the ‘optimal’ UV dosage that will (i) produce a safe product (ii) represent a germicidal efficacy similar to that of pasteurisation (iii) would limit

organoleptic defects in milk associated with ‘over-exposure’ of UV (consistent with sensory analysis conducted during the challenge testing) and (iv) would be commercially viable to use in a current medium sized cheese factory production line. Various other tests (not included in this manuscript) have been conducted in this regard, confirming the optimal UV-C dosage level for the commercial application of UV treatment used for the cold processing of raw milk. Parameters that were tested in such ‘challenge’ tests included: flow rate, UV-C dosage, residence time, different turbulent flow patterns (i.e. different Reynolds values), different micro-organisms and inoculums in milk being treated, varying milk composition, varying milk temperature and the effect of UV on germicidal efficacy and organoleptic properties.

3.4. References

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3.5. Connecting text

A comprehensive review of literature as well as the design optimization of the SurePure TurbulatorTM UV system (as discussed in Chapter 3), demonstrated the need for further studies regarding the germicidal efficacy of UV treatment in liquid milk. Part of the research was presented at the American Society of Agricultural and Biological Engineers (ASABE) Annual Conference in 2006 at the Portland Convention Centre Portland, Oregon:

Reinemann, D. J., Gouws, P. A., Cilliers, F. P., Houck, K., & Bishop, J. R. (2006). Bacterial and Sensory Evaluation of Ultraviolet Treated Raw Milk. *2006 ASABE Annual International Meeting, Paper 0660880* Portland, Oregon: Portland Convention Centre.

The research project was done in collaboration with the Department of Biological Systems Engineering and the Centre for Dairy Research of the University of Wisconsin in Madison, United States as well as the Department of Biotechnology of the University of the Western Cape in Cape Town, South Africa. Important measurable outputs of the research were the quantification of dose responses on various microorganisms using the thin-film, turbulent-flow UV treatment system (SurePure) as well as defining a less ambiguous UV-dose calculation, specifically aimed at liquid photopurification.

Chapter 4

Bacterial and sensory evaluation of ultraviolet (UV) treated raw milk

4.1. Abstract

A novel Ultraviolet (UV) system was shown to be capable of reliably achieving in excess of a 3 log reduction in bacteria measured as standard plate, psychrotrophic, coliform and thermotolerant counts. Sensory analysis indicated that excessive or improper treatment may affect milk flavour so that care must be taken in the way that UV treatment is applied. Possible applications include treatment of milk fed to calves to reduce disease transmission, cold treatment of raw milk, reduction of bacteria not susceptible to thermal treatment, psychrotrophic reduction in refrigerated milk stored for prolonged periods, and bacteria reduction to improve milk quality where lack of a reliable energy supply and high cost make on-farm refrigeration prohibitive.

Keywords: Milk quality, UV light

4.2. Introduction

The National Advisory Committee on microbiological criteria for foods lists Ultraviolet (UV) radiation as a non-thermal technology that can satisfy the definition of pasteurisation for certain foods. UV wavelengths of 200-280 nm will inactivate bacteria and viruses in liquids, such as water and fruit juices. These liquids have been successfully treated with UV light to reduce bacteria counts (Sastry *et al.*, 2000; Hanes *et al.*, 2002; Quintero-Ramos *et al.*, 2004; Wright *et al.*, 2000). The FDA has given market approval to use UV radiation for the treatment of water and food under specific conditions (CFR, 2005). Smith *et al.*, (2002) reported that, “in principle, the bacterial content of milk can be adequately controlled by exposure to pulsed ultraviolet light.” In practice, the application of UV treatment to milk has been challenging for two main reasons

- The solids content of milk limits the penetration of UV light into the liquid, thereby reducing its efficacy, and
- Excessive UV exposure can lead to oxidation and sensory defects in milk.

Some applications of UV treatment to milk have been reported, however. Matak *et al.*, (2005) reported that *Listeria monocytogenes* was reduced by a 5 log in goat's milk. In large fluid volumes pulsed UV appears to have a higher penetration depth than continuous UV (Krishnamurty *et al.*, 2004) and has been shown to inactivate bacteria in cow's milk (Smith *et al.*, 2002). Critical design elements in the application of UV treatment to milk have been identified as UV wavelength, intensity and dose rate, thickness of the radiation path and flow turbulence.

A series of bench-scale studies were conducted at the University of Wisconsin, Milking research and Instruction lab and Centre for Dairy Research to evaluate the efficacy of a novel UV treatment system that was designed to address some of the limitations of applying UV treatment to milk. Subsequent pilot scale tests were also performed at the Elsenburg Dairy Research facility of the Agricultural Research Council, Stellenbosch. The objectives of these studies were to evaluate the effectiveness of UV treatment for reducing bacteria counts in raw milk, and to measure sensory defects in milk subjected to varying levels of UV treatment.

4.3. Materials and Methods

4.3.1. UV treatment

Milk was exposed to UV irradiation using the PureUV version 1 and 2 systems (system 2 was only used in the pilot scale trial) for turbid liquids. The PureUV system is designed to increase the penetration of UV throughout the liquid being treated and to ensure maximum exposure to individual bacterial cells. The system configuration consisted of a single germicidal low-mercury UV lamp (30 UV-C Watts, 90 % 254nm and 90 % emittance) housed in a quartz sleeve. The UV lamp and quartz sleeve is encased in a corrugated stainless steel chamber, resulting in a

turbulent flow of liquid between the quartz sleeve and corrugated stainless steel chamber. Multiple passes were done in order to obtain different dosage levels that correlated with different log reductions of the bacteria analysed. The lamps were tested prior and after each run for UV output. All lamps recorded readings in excess of 350 $\mu\text{W}\cdot\text{sec}/\text{cm}^2$ at one meter, as per suppliers' specification.

The system was cleaned using standard "Cleaning In Place" (CIP) principles for dairy equipment: 1. Rinse with warm water (50°C) for 10 minutes; 2. Circulation of a 1.0% alkaline detergent solution for 30 minutes at 75°C; 3. Rinse out alkaline detergent with warm water (50°C) for about 5 minutes; 4. Circulation of 0.75% (nitric) acid solution for 20 minutes at 70°C; 5. Final rinse with cold water.

4.3.2. Bench Scale Tests

Bacteria Reduction: A series of experiments was designed to quantify bacteria reduction in raw milk due to UV treatment. In all three protocols, milk was inoculated to ensure high bacteria counts for all major categories of bacteria common in raw milk. The inoculants contained strains of bacteria that had been isolated from raw milk samples taken from Wisconsin dairy farms. Two different strains of each of the following bacteria types were used for the inoculants.

- *Streptococcus uberis*
- *S. dysgalctia*
- *S. thermophilus*
- *Lactobacillus casei*
- *Escherichia coli*
- *Staphylococcus aureus*
- *Bacillus sp.*
- *Pseudomonas sp.*
- *Enterobacteria sp.*
- *Citrobacter freundii*
- *Serratia marcesceas*
- *Enterobacter cloacae*
- *Enterobacter faecalis*

Bacteria colonies were removed from agar plates using a milk sample loop. Each loop was immersed in a container of 250 mL of milk and the loops were vigorously stirred in the milk. This solution was then allowed to incubate at room temperature for 12 hours before being added to the experimental milk samples. Treatment protocol three allowed additional incubation time to further increase bacteria counts. The bacteria counts at the start of all treatments were therefore higher than would be expected in raw milk. The following three treatment protocols, representative of on-farm treatment scenarios were investigated.

Treatment 1. UV treatment immediately after milk harvest (milk temperature 36°C). A sample of 20 litres of raw milk was collected from the UW milking parlour by milking a cow directly into a collection bucket. This milk was transported to the UW milking lab, inoculated and UV treatment was applied within ½ hour of milk collection (Initial counts: SPC 6.8×10^6 cfu.mL⁻¹ psych. 6.8×10^6 cfu.mL⁻¹, therm. 1.6×10^4 cfu.mL⁻¹, coli. 7.0×10^4 cfu.mL⁻¹). This treatment simulated a scenario in which milk might be treated ‘in-line’ as it is transferred from the receiver in a milking parlour to a bulk storage tank.

Treatment 2. UV treatment after milk refrigeration (Milk temperature 4°C). A sample of 20 litres each of raw milk was collected from the refrigerated bulk tank in the UW milking parlour. This milk was transported to the UW milking lab, inoculated and UV treatment applied about 2 hours after milk collection to allow time for the milk to be chilled to storage temperature (Initial counts: SPC 9.9×10^6 cfu.mL⁻¹, psych. 1.0×10^6 cfu.mL⁻¹, therm. 8.4×10^2 cfu.mL⁻¹, coli 2.0×10^4 cfu.mL⁻¹). This treatment simulated a scenario in which milk might be treated by recirculating cooled milk in a refrigerated bulk storage tank.

Treatment 3. UV treatment after un-refrigerated storage for 6 hours (Milk temperature 25°C). A sample of 20 litres of raw milk was collected from the UW milking parlour by milking a cow directly into a collection bucket. This milk was transported to the UW milking lab, inoculated and then allowed to incubate for 6 hours before UV treatment was applied (Initial counts: SPC 2.7×10^7 cfu.mL⁻¹, psychrotrophs 2.0×10^7

cfu.mL⁻¹, therm. 3.5×10^4 cfu.mL⁻¹, coli 2.3×10^4 cfu.mL⁻¹) simulating a situation in which refrigerated storage is not used on a farm.

UV treatment has been characterized in the literature by an energy delivered per surface area of the treatment device (e.g. mJ.cm⁻², Matak *et al.*, 2005). This characterization is difficult to apply for situations which may have very different depths of treatment liquid. An alternate treatment method is to characterize the amount of UV energy delivered per volume of milk treated as follows.

For batch operations in which the liquid is re-circulated through a reactor:

$$D_{uv} = (P_{uv} \times t) / V \quad (\text{Equation 1})$$

For single pass, continuous flow operations;

$$D_{uv} = P_{uv} / Q \quad (\text{Equation 2})$$

Where:

- D_{uv} = UV dose (Joules / liter)
- P_{uv} = UV power delivered (Watts)
= Lamp output (Watts) x Transmission efficiency
- t = Treatment time (s)
- V = Treated Volume of liquid (litres)
- Q = liquid flow rate (litres.s⁻¹)

This method of specifying the UV dose is less ambiguous than energy delivered per surface area especially when the depth of liquid film is variable. As will be shown in the following data the relationship between the UV dose rate as described above and the log reduction in bacteria count per mL was approximately linear over a broad range of bacteria counts. The slope of the regression between log bacteria count per mL and the UV dose in J.L⁻¹ was used to describe a 'kill rate' for each bacteria type (log reduction per kJ.L⁻¹ UV dose).

A sample volume of 22 litres of inoculated milk was placed into a milking machine receiver and circulated through the UV treatment unit (wavelength = 254 nm) using a speed controlled milk pump to achieve a flow rate of 1.1 l.s⁻¹. The milk receiver was placed under partial vacuum, typical of that used during milking. An in-line sampler was used to extract milk from the flow stream without stopping the treatment process to avoid excessive exposure of milk in the UV reactor during sampling. Samples were taken after 1, 2, 4, 8, 16, 32 and 64 passes of the sample volume through the UV reactor, corresponding to dose rates of 0.23, 0.46, 0.93, 1.9, 3.7, 7.4 and 15 kJ.L⁻¹.

Milk samples were analysed for standard plate count, coliform, psychrotrophs and thermotolerant bacteria. Samples were taken on the raw milk, immediately following UV exposure (UV). Duplicate milk samples were taken and each plated in duplicate. Standard plate counts were determined by plating on aerobic plate count Petrifilm (3M, St. Paul, MN) and incubating at 32°C for 48 hours. Thermotolerant bacteria counts were conducted by heating 5mL aliquots of milk samples to 62.8°C for 30 minutes and immediately cooling to 10°C in an ice bath. Cooled milk samples were then plated on aerobic plate count Petrifilm with incubation at 32°C for 48 hours. Psychrotrophic bacteria were enumerated using the modified psychrotrophic bacteria count. Milk was plated on aerobic plate count Petrifilm and incubated at 21°C for 48 hours. Coliform counts were conducted by plating samples on *E.coli*/coliform petrifilm and incubating at 35°C for 24 hours.

4.3.3. Pilot Scale tests

The UW trials used a single UV reactor (30 Watt UV output) with milk recirculated to achieve the desired dose rates. In some commercial applications it is likely that multiple UV reactors would be used in series to increase the UV dose rate for a single pass of milk. Bacteria reduction studies were therefore performed at the Elsenburg Dairy Research Facility (Agricultural Research Council, South Africa) using a larger volume of milk and a treatment system (Pure UV unit) consisting of 10 UV reactors connected in series (300 Watt UV output). Freshly drawn cooled raw milk was collected from the milking parlour and transported to the dairy laboratory.

The milk was either treated directly at a temperature of about 4°C or was heated in a double jacketed steam tank (batch pasteuriser) to approximately 37°C and then UV treated. Each treatment used 200 litres of milk that was pumped between two tanks at a flow rate of 1 s^{-1} . Samples were aseptically drawn from the initial batch of milk and after each pass through the 10 reactor UV system. Samples were immediately cooled to less than 5°C and stored for microbiological analysis immediately after UV treatment and again after 24 hours at the University of the Western Cape.

Milk samples were analysed for standard plate count, *E.coli*/coliform, psychrotrophs and spore forming bacteria. Samples were taken on the raw milk, immediately following UV exposure (UV). Duplicate milk samples were taken and each plated in duplicate. Aerobic plate counts were determined by plating on aerobic plate count Petrifilm (3M, St. Paul, MN) and incubating at 32°C for 48 hours. Psychrotrophic bacteria were enumerated using the modified psychrotrophic bacteria count. Milk was plated on aerobic plate count Petrifilm and incubated at 21°C for 48 hours. Coliform counts were conducted by plating samples on *E.coli*/coliform Petrifilm and incubating at 35°C for 24 hours using the same Petrifilm analysis methods as used in the UW studies as well. In addition assessments were made of spore forming bacteria and *E. coli* for several trials.

In addition one set of samples were incubated at 4°C for 24 hours after UV treatment to investigate the possibility of photo-reactivation and photolyase DNA repair within bacterial cells.

4.3.4. Sensory Testing

A series of experiments were done to investigate the level of UV treatment that might affect sensory quality of milk. Raw bulk tank milk from the University of Wisconsin Dairy Plant was collected in sanitized stainless steel containers, stored at 4°C, and used for experiments within 24 hours. Raw milk was recirculated from the collection tank through the UV reactor (254 nm UV wavelength) at a flow rate of 1.1 L s^{-1} to achieve dose levels of 0.47, 0.93 and 1.5 kJ L^{-1} corresponding to a 1-, 2- and 3-log reduction in SPC. Approximately 10 L each of the raw milk sample and milk exposed

at varying UV dosages were pasteurised (74°C for 15 seconds) using a small plate heat exchanger with holding loop. The pasteurised milk was then collected in aseptic 500 mL bottles and stored for the duration of the study in a lighted cooler at 4°C. Sensory evaluations were performed by trained panellists in accordance with the guidelines of the American Dairy Science Association (ADSA) as described by Bodyfelt et.al. (1988) at 1, 7, 14, and 21 days after bottling. Personnel serving as panellists differed throughout the evaluation period. At the end of each evaluation, panellists were asked which sample they preferred. Samples were taken from the pasteurisation alone (HTST- control), UV treatment alone (UV) and after UV plus pasteurisation (HUV) and analysed for bacterial analysis as described above at the time of bottling and at the time of each sensory evaluation.

4.4. Results

4.4.1. Bacteria Reduction Results

Example data sets from the UW bench scale studies (Figure 4.1) and the Elsenburg pilot scale studies (Figure 4.2) are presented in Figures 4.1 & 4.2 respectively. The kill rates (log reduction per kJ.L^{-1} of UV dose) for each experiment is summarized in Table 4.1. An analysis of variance (ANOVA) indicated the effect of treatment temperature on the kill rate in the UW studies was not significant ($p = 0.49$) however the difference between bacteria types was significant ($p = 0.03$). Likewise the effect of temperature was not significant for the Elsenburg tests ($p = 0.13$) and the difference between bacteria types was ($p = 0.02$). An additional ANOVA with data for the common bacteria tests (SPC, psychrotrophs and coliforms) for the UW and Elsenburg tests indicated that there was not a significant difference between the bench scale and pilot scale tests ($p = 0.68$). The average of all test temperature and location can therefore be presented in Table 4.1 for each of the bacteria types.

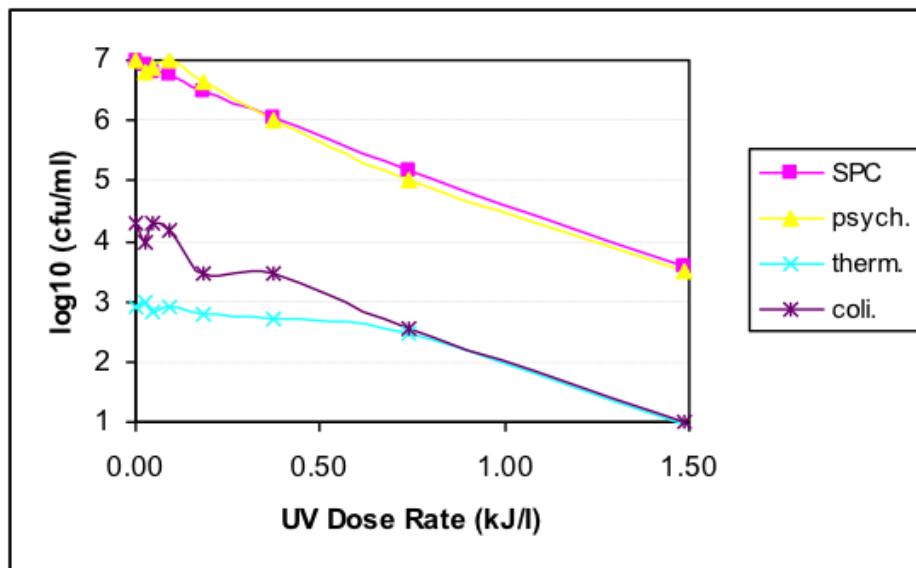


Figure 4.1: Bacteria count versus UV treatment dose for inoculated raw milk incubated raw milk for UW treatment 3, inoculated and refrigerated milk (4°C) treated at 2 hours after milking.

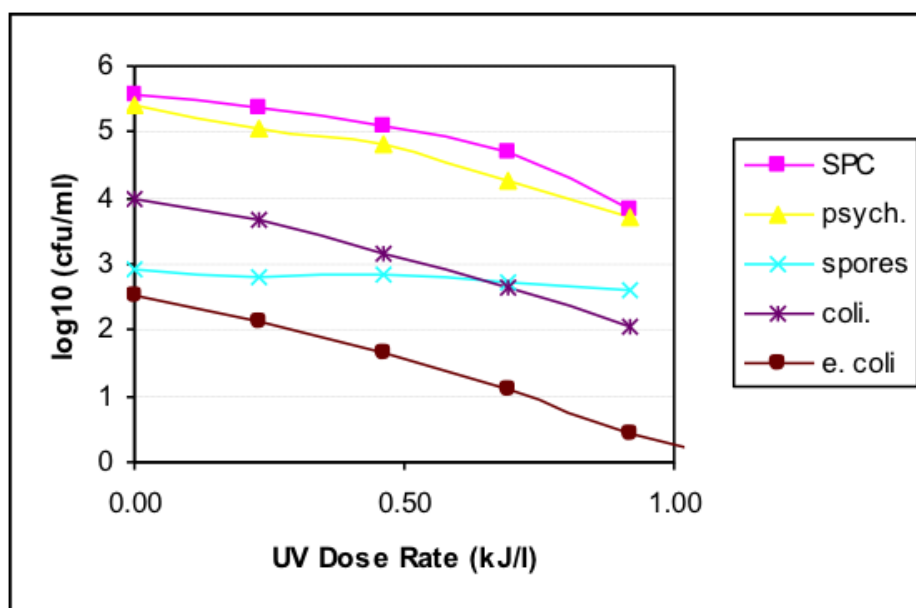


Figure 4.2: Bacteria count versus UV treatment dose for inoculated raw milk incubated raw milk for Elsenburg test, inoculated and refrigerated milk (4°C) treated at 2 hours after milking.

Table 4.1: UV bacteria kill rate expressed as log reduction per kJ.L^{-1} UV dose \pm standard error of estimate and (R-square) for different treatment temperatures and bacteria types. UW - Bench scale tests recirculated milk through a single UV reactor. Elsenburg - Pilot scale tests used 10 UV reactors in series.

Test Type	Temperature	SPC	psych.	therm.	coli	<i>E. coli</i>	spores
UW – Bench	38 °C	1.51 +/- 0.15 (0.95)	1.88 +/- 0.17 (0.95)	1.67 +/- 0.27 (0.88)	2.32 +/- 0.36 (087)	-	-
UW – Bench	25 °C	2.91 +/- 0.17 (0.98)	1.73 +/- 0.18 (0.94)	1.36 +/- 0.01 (0.99)	2.75 +/- 0.13 (0.97)	-	-
UW – Bench	4 °C	2.29 +/- 0.04 (0.99)	2.41 +/- 0.10 (0.99)	1.25 +/- 0.14 (0.93)	2.19 +/- 0.14 (0.99)	-	-
Elsenburg – Pilot	38 °C	1.27 +/- 0.15 (0.96)	2.55 +/- 0.25 (0.97)	-	3.18 +/- 0.46 (0.94)	3.05 (n=2)	0.43 +/- 0.37 (0.31)
Elsenburg – Pilot	4 °C	1.81 +/- 0.32 (.91)	1.83 +/- 0.17 (0.97)	-	2.12 +/- 0.11 (0.99)	2.02 +/- 0.15 (0.97)	0.29 +/- 0.07 (0.86)
Average		1.92	2.08	1.42	2.48	2.53	0.36

4.4.2. Sensory Evaluation Results

The results of the sensory evaluation are summarized in Tables 4.2 and 4.3. For each trial, “light oxidized” was the most noted defect for each type of milk. The off-flavour was noted 55% of the time in both the control and experimental treatments. This defect can be attributed to the fact that samples were stored in a lighted cooler during the duration of the study. These conditions are similar to that found in a majority of supermarkets today. The “flat off-flavour” is one of the most commonly detected in skim milk according to Bodyfelt *et.al*, (1988) and was seen in 40% of samples evaluated in these trials.

Table 4.2. Average sensory score (a score of 10 represents samples with no defects noted) and overall preference (percentage of panellist preferring one sample more) for milk samples treated by pasteurisation alone (HTST) and pasteurisation + UV treatment (HUV) (NT = not tested).

Days after treatment	Test 1.			Test 2.			Test 3.		
	HTST	HUV (0.47 kJ.L ⁻¹)	Pref.	HTST	HUV (0.93 kJ.L ⁻¹)	Pref.	HTST	HUV (1.4 kJ.L ⁻¹)	Pref.
1	8	9	H+UV (66%)	3	4	No Pref. (50%)	7	5	HTST (100%)
7	6	7	HTST (33%)	7	6	HTST (80%)	7	5	HTST (75%)
14	7	8	H+UV (80%)	6	6	HTST (33%)	7	5	HTST (100%)
21	8	9	H+UV (33%)	NT	NT	NT	NT	NT	NT

Table 4.3: Detected off-flavours (percentage of times off-flavour noted) for milk samples treated by pasteurisation alone (HTST) and pasteurisation + UV treatment (HUV).

Days after treatment	Test 1.		Test 2.		Test 3.	
	HTST	HUV (0.47 kJ.L ⁻¹)	HTST	HUV (0.93 kJ.L ⁻¹)	HTST	HUV (1.4 kJ.L ⁻¹)
1	Flat (50%)	Flat (50%)	Light oxidized (75%)	Unclean (50%)	Light oxidized, flat (33%)	Cooked, rancid, barny (33%)
7	Light oxidized (60%)	Light oxidized, feed, flat (40%)	Light oxidized, cooked (50%)	Light oxidized, cooked, flat, unclean (33%)	Light oxidized, flat (40%)	Flat, unclean (40%)
14	Light oxidized (50%)	Light oxidized (50%)	Light oxidized (80%)	Feed (60%)	Light oxidized (80%)	Unclean (60%)
21	Feed (60%)	Flat, feed (40%)	NT	NT	NT	NT

A majority of samples evaluated (75%) had scores >6. Carey *et. al* (2005) noted that scores < 6 using the ADSA style format were considered unacceptable had potential to warrant consumer complaints. Low scores were a factor in milk exposed to the 1.4 kJ.L⁻¹ UV dose. Scores for HUV samples during the 14 day testing period were never higher than 6, with panellist preferring HTST milk 92% of the time. The most

common off-flavour noted in HUV milk at the 1.4 kJ.L^{-1} dose was unclear. Milk exposed to UV at a dose of 0.47 J.L^{-1} was preferred at 1 and 14 days over HTST, with slight preference to HTST on days 7 and 21. Scores for off-flavours between 0.47 kJ.L^{-1} HUV and HTST samples were always within 1 point throughout 21 day shelf-life. The same is true of HUV exposed at 0.93 kJ.L^{-1} and HTST samples through 14 days shelf-life, however overall scores were generally lower for this trial.

The bacterial quality of milk during shelf-life did not change significantly, regardless of treatment or UV dosage. No coliforms were detected during the study. Both HTST and HUV milks remained below 50 cfu.mL^{-1} for Standard Plate Count during the duration of shelf-life. All bacterial counts, on average, were lower for milk exposed to UV light and HTST than those treated with pasteurisation alone. There was therefore no evidence that sensory defects were related to microflora of the milk.

4.5. Discussion and Conclusions

The method used to describe UV treatment levels in this paper was found to be a good predictor of bacteria reduction across bench and pilot scale applications across a wide range of treatment levels and bacteria counts. UV treatment was shown in these studies, to be capable of reducing standard plate count, psychrotrophic, coliform, *E. coli*, and thermophilic spore forming bacteria counts in raw milk. The reduction efficacy was best for coliforms followed by psychrotrophs and thermophiles with modest reductions in spore forming bacteria. Sensory panels showed a preference for the UV treated milk when treated to achieve a 1-log (10x) reduction in bacteria for storage intervals up to 21 days. Sensory panellists found little difference between milk that was UV treated to achieve a 2-log (100x) reduction for storage intervals up to 21 days. Changes in flavour were observed in milk treated to achieve 3-log (1000x) reduction in standard plate counts. Excessive or improper treatment may, therefore, affect sensory qualities of milk so that care must be taken in the way that UV treatment is applied for various milk products.

Possible applications of this technology include cold treatment of raw milk, reduction of bacteria not susceptible to thermal treatment, psychrotrophic reduction in

refrigerated milk stored for prolonged periods, and bacteria reduction to improve milk quality in parts of the world where lack of a reliable energy supply and high cost make on-farm refrigeration prohibitive.

4.6. Acknowledgements

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4.8. Data Appendix

Table 4.4: Summary of results of bench scale bacteria reduction experiments (Slope term = Log reduction in cfu.mL⁻¹ per kJ.L⁻¹ dose).

Treatment 1. Warm milk (38°C) Inoculated and treated at milking time.				
UV dose (J.L ⁻¹)	Log (SPC)	Log (Psych.)	Log (Therm.)	Log (Coli)
0	6.83	6.83	4.19	4.85
23	6.43	6.48	4.17	5.15
46	6.40	6.39	3.78	4.54
93	6.26	6.18	3.73	4.00
185	6.06	5.89	3.66	4.08
371	5.76	5.49	3.20	3.15
742	5.15	4.86	2.89	2.28
1484	4.38	3.83	-	1.54
Slope term	1.51	1.88	1.67	2.32
<i>Std. Err. Slope</i>	0.15	0.17	0.27	0.36
R Square	0.95	0.95	0.88	0.88
Treatment 2. Inoculated milk Incubated at 25°C for 6 hours.				
UV dose (J.L ⁻¹)	Log (SPC)	Log (Psych.)	Log (Therm.)	Log (Coli)
0	7.44	7.31	-	6.36
23	7.61	7.18	-	6.48
46	7.30	-	-	6.42
93	7.08	7.04	-	6.23
185	6.94		-	5.85
371	6.49	6.96	4.03	5.34
742	5.81	5.72	3.54	4.04
1484	3.00	4.85	2.53	2.52
Slope term	2.91	1.73	1.36	2.75
<i>Std. Err. Slope</i>	0.17	0.18	0.01	0.13
R Square	0.98	0.94	0.99	0.99
Treatment 3. Refrigerated Milk (4°C) Inoculated and treated 2 hours after milking				
UV dose (J.L ⁻¹)	Log (SPC)	Log (Psych.)	Log (Therm.)	Log (Coli)
0	7.00	7.01	2.92	4.30
23	6.90	6.81	3.00	4.00
46	6.78	6.88	2.81	4.30
93	6.77	6.99	2.90	4.18
185	6.48	6.62	2.80	3.48
371	6.04	6.01	2.71	3.48
742	5.18	5.03	2.46	2.54
1484	3.57	3.52	0.98	1.00
Slope term	2.29	2.41	1.25	2.19
<i>Std. Err. Slope</i>	0.04	0.10	0.14	0.14
R Square	0.99	0.99	0.93	0.99

Table 4.5: Summary of Elsenburg Pilot Scale Studies (Slope term = Log reduction in cfu.mL⁻¹ per kJ.L⁻¹ dose).

Treatment 1: Refrigerated Milk (4°C) Elsenburg				
UV dose (J.L ⁻¹)	Log (SPC)	Log (Psych.)	Log (Spores)	Log (Coli)
0	5.56	5.40	2.94	4.00
230	5.37	5.07	2.79	3.67
459	5.09	4.82	2.83	3.16
689	4.68	4.25	2.74	2.65
918	3.83	3.71	2.63	2.07
Slope term	1.81	1.83	0.29	2.12
R Square	0.91	0.97	0.87	0.99
Intercept	5.73	5.49	2.92	4.08
<i>Std. Err. Slope</i>	0.32	0.17	0.07	0.11

Treatment 2: Warm Milk (37°C) Elsenburg				
UV dose (J.L ⁻¹)	Log (SPC)	Log (Psych.)	Log (Spores)	Log (Coli)
0	5.34	5.00	1.89	2.76
230	5.31	4.49	1.93	1.79
459	4.87	3.84	1.84	0.94
689	4.515	2.985	1.24	0
918	4.28	2.82	1.735	0.00
Slope term	1.27	2.55	0.43	3.97
R Square	0.96	0.97	0.31	0.99
Intercept	5.44	4.99	1.93	2.74
<i>Std. Err. Slope</i>	0.15	0.25	0.37	0.05

Treatment 3: Refrigerated Milk (4°C) Elsenburg, analysed after 24 h incubation at 4°C		
UV dose (J.L ⁻¹)	Log (SPC)	Log (Coli)
0	5.09	2.51
230	4.80	2.13
459	4.71	1.66
689	4.35	1.11
918	4.06	0.45
1150	3.74	0
1380	3.05	0
Slope term	1.38	2.02
R Square	0.95	0.97
Intercept	5.21	2.52
<i>Std. Err. Slope</i>	0.14	0.15

Treatment 4: Refrigerated Milk (4°C) Elsenburg, treated with modified, reduced flow-path

UV dose (J.L ⁻¹)	Log (SPC)	Log (Psych.)	Log (Coli)
0	4.90	4.82	2.22
328	4.87	4.74	1.74
656	4.32	4.07	1.07
984	3.65	3.44	1.00
1020	3.46	3.14	0.00
1639	3.25	2.90	0.00
1967	1.91	2.39	0.00
Slope term	1.45	1.30	1.44
R Square	0.92	0.94	0.85
Intercept	5.13	4.87	2.11
Std. Err. Slope	0.19	0.15	0.30

4.9. Connecting Text

Following the elucidation of the germicidal efficacy on milk described in Chapter 4, using the SurePure thin-film, turbulent-flow UV treatment system, further studies were conducted to quantify and qualify the effect of UV radiation on the chemical and biochemical compounds in milk. In part 1 of the study, as described in Chapter 5, milk was exposed to 1 kJ.L⁻¹ of UV-C radiation using a commercial UV system (SurePure SP-40) and the results of the chemical and biochemical analysis were compared to conventional pasteurisation and a combination of UV treatment and pasteurisation. The research results were published in a peer-reviewed scientific journal *Innovative Food Science and Emerging Technologies*:

Cilliers, F.P., Gouws, P.A., Koutchma, T., Engelbrecht, Y., Adriaanse, C. & Swart, P. (2014). A microbiological, biochemical and sensory characterization of bovine milk treated by heat and ultraviolet (UV) light for manufacturing Cheddar cheese. *Innovative Food Science and Emerging Technologies*, 23, 94–106.

Furthermore the milk processed with each of the treatments was used for the manufacture of Cheddar cheese (Phase 2 of the study) as described in Chapter 6.

Chapter 5

Microbiological, biochemical and sensory characterization of bovine milk treated by heat and ultraviolet (UV) light for the manufacturing of Cheddar cheese

5.1. Abstract

The aim of the study was to quantify microbiological, biochemical and sensory changes in full cream raw milk (RM) processed with high temperature short time (HTST) pasteurisation (P), ultraviolet light treatment (UV) and a combination of UV light treatment and HTST pasteurisation (UVP) in commercial scale production of Cheddar cheese. The three treatments have been compared at a similar level of microbial efficacy. No significant differences were reported on the macronutrient composition, however a 35% and 18% reduction in cholesterol in the UV and UVP treatments were observed. HTST treatment (UVP and P) reduced riboflavin and vitamin B₁₂ in milk by ~31% and ~18% respectively, with no reduction in riboflavin and vitamin B₁₂ observed after the UV treatment alone. Lipid oxidation and lipolysis results indicated a significant difference between raw and UVP treated milk ($p < 0.05$). Protein oxidation results indicated no significant differences except for methionine sulfone, which increased by 67.2% and 87.9% respectively for UV and UVP treatments. Some differences were noted with sensory results, most noticeably on the 'tallowy' flavour descriptor for the UV treated milk, however customer acceptance of UV treated milk will ultimately determine the acceptability of UV technology as an alternative or adjunct to commercial thermal treatment of milk in cheese production.

Keywords: Ultraviolet (UV) treatment of cheese milk; UV treatment followed by HTST; Turbulent flow commercial UV system; Microbial reduction; Milk composition; Biochemical effects on lipids and protein

Industrial relevance: UV light has been proposed as a non-thermal alternative or adjunct to traditional heat treatment for the reduction of microorganisms in fluids. UV technology presents numerous benefits over traditional preservation, such as use of heat treatment, for example: low cost of installation and maintenance, lower production cost and the reduction of carbon emissions when compared to traditional thermal pasteurisation systems. Furthermore UV technology also offers alternative processing technology in developing countries where production of milk and cheese are done on a small scale. Known limitation for the efficacy of UV as a processing option was its low penetration depth into turbid liquids, such as milk, and the possible negative impact on organic compounds when over-exposed to UV light. New optimised reactor design features of the swirl tube SurePure TurbulatorTM, could potentially negate these negative effects associated with UV treatment due to improved uniformity of treatment. This technology would provide an affordable and accessible processing solution to not only enhance the safety and extend shelf-life of the milk being produced, but will also increase final product quality of secondary dairy products such as Cheddar cheese produced from UV treated milk. These advantages could positively impact on the safety, profitability and sustainability of the agro-industrial sector within such regions.

5.2. Introduction

Food safety is of paramount importance to the dairy sector, which includes the overall quality of milk (as a primary product) and the quality of secondary dairy products produced from milk, such as cheese. Healthy cows' milk contains relatively few bacteria (10^2 – 10^3 cfu.mL⁻¹) just after milking; however, milk remains a nutrient dense natural food that can be contaminated easily post-milking. Raw milk has a varied microflora population arising from several sources, contamination post-milking introduced by various factors, such as the exterior surfaces of the animal and/or surfaces of milk handling equipment (i.e. milking machines, pipelines, and containers). As a result, milk is susceptible to contamination by many pathogenic microorganisms with an additional potential risk of transferring diseases from the animals to humans (i.e. tuberculosis, brucellosis, typhoid fever, and listeriosis). Thermal pasteurisation is traditionally used to reduce the number of pathogens to such

an extent that the processed milk no longer poses a health risk when consumed (Smith, Lagunas-Solar, & Cullor, 2002). In addition pasteurisation is an important processing step that will also prolong product shelf life. The effect of heat treatment is well documented and various studies have quantified the effects of pasteurisation and heat treatment on the nutritional and organoleptic quality of milk (Walstra & Jenness, 1984). The most important changes associated with heat treatment of milk is a decrease in pH, partial precipitation of calcium phosphate, denaturation of whey proteins and interaction with casein, lactose isomerisation, Maillard browning and modifications to the casein micelle. The resulting effects, besides making it safe for consumption and increasing shelf-life, are associated changes to the sensory characteristics, such as appearance, colour, flavour and texture and minor changes to the nutritional value of the milk being treated. Pasteurised milk contains 10^3 – 10^4 cfu.mL⁻¹ APC following pasteurisation, depending on the factors mentioned above. The initial microbiological quality is not only important from a food safety point of view, but also important when using milk for further processing to limit secondary defects that might occur due to high initial bacterial counts which could lead to secondary products of inferior quality (Burton, 1986; Kessler, 2002).

At present, there is growing interest in alternative non-thermal pasteurisation and shelf-life extension technologies (ESL), such as ultraviolet (UV) radiation (Donaghy *et al.*, 2009; Keyser, Müller, Cilliers, Nel, & Gouws, 2007; Koutchma, Keller, Chirtel, & Parisi, 2004, Koutchma, Paris, & Patazca, 2007; Matak *et al.*, 2005; Reinemann, Gouws, Cilliers, Houck, & Bishop, 2006). UVC germicidal wavelengths of 200–280 nm inactivate bacteria and viruses and have been used to successfully reduce microbial counts in water, fresh juices and on non-food contact and food contact surfaces (Hanes *et al.*, 2002; Quintero-Ramos, Churey, Hartman, Barnard, & Worobo, 2004; Sastry, Datta, & Woroboo, 2000; Wright, Summer, Hackney, Pierson, & Zoecklein, 2000). The US FDA has given market approvals to use UV radiation for the treatment of food surfaces, water and fresh juice products under specific conditions defined by CFR (CFR, 2005). The UV germicidal effect is based on the absorption of light photons by exposed cells and damage the DNA by causing thymine dimer (peptide bonds) formation, thus destroying the microorganisms' ability to grow (Guerrero-Beltrán & Barbosa-Cánovas, 2004).

According to Koutchma (2009), UV treatment presents numerous benefits over traditional thermal technologies that include the relatively low initial investment cost, low maintenance and production costs and the significantly less carbon emissions. Some of the potential applications of UV technology recently identified include cold treatment of raw milk. For example, UV light can not only enhance reduction of bacteria and spores that are not susceptible to thermal pasteurisation, but also reduce the number of psychrotrophic bacteria in milk that has to be stored for prolonged periods therefore improving milk quality in developing countries where lack of a reliable energy supply and high cost make on farm refrigeration prohibitive (Krishnamurthy, Demirci, & Irudayaraj, 2004, 2007; Koutchma, 2009; Nazarowec-White & Faber, 1997). Even though UV treatment of milk has been challenging, due to opaqueness or extremely low UV transmittance (UVT), a few applications of UV in milk have been reported. Matak *et al.* (2005) reported that *Listeria monocytogenes* was reduced by a 5 log in goat's milk using ultraviolet fluid processor (CiderSure 3500A, FPE Inc., Rochester, NY) at a targeted UV dose of $15.8 \pm 1.6 \text{ mJ.cm}^{-2}$. Reinemann *et al.* (2006) applied a novel UV treatment system (PureUV, Milnerton, South Africa) and reported that UV treatment at a targeted UV dose of 1.5 kJ.L^{-1} was capable of reducing standard plate, psychrotrophic, coliform, *E. coli*, and thermotolerant spore forming bacteria counts in raw milk. The reduction efficacy was the highest for coliforms (~3 log) followed by psychrotrophs and thermotolerants (~2 log) with modest reductions in spore forming bacteria (0.5 log) without changing the sensory character of the milk. Critical process parameters in continuous flow UV treatment of fluids have been identified as UV wavelength, applied and absorbed UV dose, thickness of the light path, fluid UVT and viscosity, and flow mixing due to turbulence (Simmons *et al.*, 2011). Even though UV irradiation has shown to be capable of reducing microorganisms in milk, the challenge remains because of any excessive UV exposure can lead to photo-oxidation and sensory defects. Optimisation of design elements in UV system, flow conditions and applied UV dose are critical for microbial efficacy and quality considerations.

In this study a SP-40 SurePure TurbulatorTM commercial system from SurePure AG, Switzerland was used for milk treatment. The SP-40 according to the manufacturer has been designed to optimise UV delivery of fluids with low UVT. The hydrodynamic performance of the SurePure TurbulatorTM has been well studied by

Simmons *et al.* (2011). It was demonstrated that the flow within the SurePure TurbulatorTM comprises of a complex swirling flow which is a function of flow-rate and allows radial mixing. The complex swirling flow associated with the system transfers UV energy directly and efficiently to the fluid in order to enhance germicidal efficacy and to limit secondary changes in the biochemical composition and the nutritional value of the milk in this instance.

The advantages of non-thermal microbial inactivation achieved with UV treatment of raw milk, can be extended further in the production of secondary dairy products, such as Cheddar cheese. The hypothesis is the character of the cheese manufactured from UV treated milk, will more likely resemble the character of cheese produced from raw milk. The UV treatment potentially limits inactivation of enzymes, such as native lipases and proteases in the milk, as well as the denaturing of the whey proteins, α -lactalbumin and β -lactoglobulin, which are changed after conventional heat treatment. In addition, UV can also limit defects occurring in the cheese associated with high initial bacterial counts in raw milk such as higher concentration of proteinases and lipases, reduction of flavour defects (fruity, stale, bitter, putrid, rancid) and achieve higher yield due to the reduction of psychrotrophic bacteria in milk from on-farm systems (Richardson & TeWhaiti, 1978). The use of UV cannot only enhance the safety of the finished product, but also allow for the reduction in final product non-conformances and achieving shorter ripening periods of cheese, however, limited research has been done to quantify the effects of UV treatment of milk on final cheese quality from a biochemical and chemical perspective. This study focused on two major objectives. First, to compare effects of conventional heat pasteurisation (P), non-thermal UV treatment (UV) and a combination of the two treatments (UVP) on raw milk in a commercial scale cheese production (part 1); Second, to compare the biochemical changes and characteristics of Cheddar cheese, manufactured from the milk processed by heat pasteurisation and UV light, and a combination of the two technologies (part 2). The goals of part 1 are to quantify microbiological effects, and to characterise biochemical and chemical changes occurring in raw milk composition as the result of the microbiologically equivalent treatments by heat and UV light.

5.3. Materials and Methods

5.3.1. Milk

Raw, full-cream milk (RM) (chilled, co-mingled morning and evening's milk from mixed Friesland and Jersey herds) were used for the manufacture of the Cheddar cheese and was collected from 3 different farmers supplying milk to a commercial cheese plant in Ashton, Western Cape, South Africa.

Upon reception, the milk was off-loaded from a tanker and cooled to under 6 °C through a Plate Cooler (Filmatic, Paarl, South Africa) and stored in a raw milk reception silo before being processed at the commercial cheese plant within 24 h of milking. In each instance 4000 L of full cream raw milk was processed to produce Cheddar cheese.

5.3.2. Milk Treatments

Three different treatments were used to process the RM before the manufacturing of the Cheddar cheese.

Treatment 1 (thermal pasteurisation, P) was used as the control: full-cream milk was thermally pasteurised through a conventional plate heat exchanger (PHE) (Filmatic, Paarl, South Africa) at 73.5 °C for 15 s and then transferred to the cheese vat at 32 °C before culture inoculation.

Treatment 2 (non-thermal UV): full-cream milk was treated with the commercial SP-40 UV system (SurePure AG, Switzerland) below 6 °C after which the milk was heated through a PHE to 32 °C and transferred to the cheese vat at 32 °C before culture inoculation.

Treatment 3 (UVP): full-cream milk was treated with the SP-40 UV system below 6 °C followed by immediate thermal pasteurisation through a conventional PHE at 73.5 °C for 15 s and transferred to the cheese vat at 32 °C before culture inoculation.

Samples of RM and after each treatment were analysed before inoculation of the cheese milk.

5.3.3. The novel turbulent flow UV-C system (SurePure Turbulator™)

The commercial SurePure 40 Turbulator™ system (SP-40), designed and manufactured by SurePure AG, Switzerland, was used in this study. The system consists of 40 single “turbulators” connected in series. The single UV turbulator applies a novel swirl tube design (‘thin film’ or ‘surface refreshment’ design features) and consists of a stainless steel inlet and outlet chamber, with a corrugated spiral tube connecting the inlet and outlet chambers. The tangential inlet of the reactor creates a high velocity and turbulence in the inlet chamber and brings the whole volume of the liquid into the contact with the UV photons. Inside the spiral tube a low-pressure mercury UV-C lamp, protected by a quartz sleeve, is housed (100 W output; 29 W UV-C output, First Light, Poultney, VT, USA). The UV light intensity on the surface of the quartz sleeve was 17.7 mW.cm^{-2} as measured by the lamp manufacturer, using calibrated radiometer. The liquid flows in the gap between the corrugated spiral tube and the quartz sleeve at a minimum flow rate (F_r) of 4000 L.h^{-1} (1.11 L.s^{-1}) with a Reynolds value (Re) in excess of 7000, indicating turbulent flow pattern as confirmed by Simmons *et al.* (2011) and the equipment manufacturer.

A single UV turbulator delivers a total applied UV-C dosage of 26.12 J.L^{-1} per volume of fluid calculated as follows:

5.3.4. Cleaning of the SP-40 system

The SP-40 system was cleaned before and after every milk treatment using standard ‘Cleaning in Place’ (CIP) procedures.

5.3.5. Sampling methodology

Triplicate milk samples (100 mL) of the three different treatments, UV and UVP as well as the raw, full-cream milk (RM) were aseptically drawn and kept on ice below 4 °C for microbiological analysis within 12 h of sampling. The samples for chemical and biochemical analysis were immediately frozen in liquid nitrogen and kept at -40 °C until analysed (within one month from sampling).

5.3.6. Milk microbial analysis

Milk and cheese samples were plated in triplicate (using serial dilutions 10^{-1} to 10^{-3}) in accordance to methods described in “*Standard Methods for the examination of Dairy Products*”, (Wehr & Frank, 2004).

5.3.7. Milk chemical and biochemical analysis

Unless otherwise specified all the tests on the milk were performed according to the standards prescribed in “*Standard Methods for the examination of Dairy Products*”, (Wehr & Frank, 2004).

5.3.7.1. Extraction of lipids and FFA from milk

Lipids and free fatty acids (FFA) were extracted from the milk according to the procedure of De Jong & Badings (1990). A 10 mL milk sample was thoroughly mixed with 10 mL of ethanol (96%) and 1.0 mL of 2.5 M H_2SO_4 for 30 min. Subsequent extraction was carried out with a 15 mL ether/heptane (1:1 ratio, v/v) in a 100 mL screw-capped centrifuge tube and then centrifuged at 4000 x g for three minutes at 20 °C. The solvent layer was transferred to a 100 mL conical flask containing 1 g anhydrous Na_2SO_4 to adsorb residual water. The extraction procedure was repeated three times with 15 mL ether/heptane (1:1 ratio, v/v).

5.3.7.2. *Fatty acid analysis*

For the analysis of the fatty acids (FA), the lipid fractions were transesterified to methyl esters in a sodium methylate solution (2 g methanol.L⁻¹). Analysis of the fatty acid methyl esters was carried out with a gas chromatograph (Hewlett-Packard, Sydney, Australia) using an FFAP-column (df 1.0 µm), helium as carrier gas, and flame ionization detection (injector temperature 250 °C; detector temperature 300 °C). The column temperature after injection of the sample was gradually increased at a rate of 10 °C.min⁻¹ from 40 °C to 250 °C and external standards were used to quantify and identify the FA.

5.3.7.3. *Free fatty acids analysis*

The free fatty acids (FFA) were determined by titration according to the AOAC official method of analysis 947.07. FFA's were determined by titration of the sample with 1 M NaOH in ethanol (95%) using phenolphthalein as indicator.

5.3.7.4. *Cholesterol and cholesterol oxides*

The trimethylsilyl (TMS) derivatisation of cholesterol and cholesterol oxides were carried out according to the method used by Pie, Spaphis, and Seillan (1990). Analyses of derivatized sterols (trimethylsilylated cholesterol and cholesterol oxides) were carried out on a Hewlett- Packard, gas chromatograph using a RTX-5 capillary-column (df 0.25 µm), helium as carrier gas, and flame-ionization detection (injector temperature 150 °C; detector temperature 330 °C). One microliter of the derivatized sample was injected at a split ratio of 50:50 onto the capillary column. The flow rate was set at 1.0 mL.min⁻¹ of He carrier gas. The cholesterol oxidation products (COPs) peaks were identified by comparison to the retention time of the reference standard. The COPs content of milk and milk product samples was determined using the internal standards (IS), 5α-cholestane and 7-ketocholesterol, the units of measurement were expressed as µg.g⁻¹ for the COPs and as percent (%) for the cholesterol.

5.3.7.5.Thiobarbituric acid reactive substances (TBARs)

The measurement of thiobarbituric acid reactive substances (TBARs) was performed using a slight modification of the method originally developed by King (1962). The milk (20 mL) was pre-heated to 30 °C. After heating milk samples were precipitated by the addition of 1.5 mL of 1 g.mL⁻¹ trichloroacetic acid (TCA) and 2 mL of 95% ethanol. Following 5 min of incubation at 30 °C, the precipitate was removed by filtration through a Whatman 3 filter paper, and 1.45% 2-thiobarbituric acid solution (1 mL) was added to the resulting filtrate (4 mL). The filtrate–TBA mixture was then incubated for 60 min at 80 °C, cooled, centrifuged at 4000 g and the absorbance was read at 531 nm. Results are shown in milligrams of malonaldehyde (MA) per kilogram (ppm). Quantification was performed using a calibration curve with tetraethoxypropane (TEP) as standard for MA.

5.3.7.6.Acid degree value (free fatty acids)

The extent of oxidative rancidity (lipolysis) in the milk was estimated by the analysis of the acid degree value (ADV) by the BDI-method and expressed as millilitre of 1 N KOH.100 g⁻¹ of fat (Singsaas & Hadland, 1972).

5.3.7.7.Absorbance at 280 nm

The TCA absorbance test for milk as per Kwan, Nakai, and Skura (1983) was followed. In this instance 10 mL milk was added to 5 mL of 30% TCA and mixed thoroughly. The reaction mixture was then incubated at room temperature for a further 5 min, and then filtered through Whatman Nr. 2 or DNA filter paper. The mixture was then diluted to a ratio of 1:5 (v/v) with distilled H₂O, and the absorbance was read at 280 nm.

5.3.7.8. *TNBS method for detection of α -amino acids*

For the determination of the α -amino acids the method of Kuchroo, Rahilly, and Fox (1983) was followed. Two and a half mL of 30% trichloroacetic acid (TCA) and 5 mL of milk was mixed and incubated at room temperature for 5 min followed by filtration through a Whatman Nr. 2 filter paper. The resulting filtrate (0.2 mL) was then mixed with 2 mL of 0.1 M borate buffer, pH 9.7 followed by the addition of 1 mL of 4 mM trinitrobenzenesulfonic acid (TNBS). After mixing the resultant mixture was incubated at room temperature for 30 min. The reaction was stopped after the 30 min of incubation by addition of 1 mL 2 M NaH_2PO_4 containing 18 mM Na_2SO_3 . The absorbance was read at 420 nm. For internal standards a dilution series of 2 M glycine (Sigma-Aldrich, SA) stock solution (0.4–2.0 mM) was prepared.

5.3.7.9. *Methionine oxidation (free and bound methionine sulfoxide and sulfone)*

The determination of methionine, methionine sulfoxide and methionine sulfone was done by EZ:Faast derivatization and solid phase extraction (Neuman, 1967). EZ:Faast consists of a solid phase extraction step followed by a derivatisation step and a liquid–liquid extraction step to remove interfering compounds in the matrix. In the solid phase extraction step the samples are passed through a sorbent tip that binds the amino acids, while the remaining interfering compounds are eluted. The amino acids are then eluted and derivatised, allowing them to migrate to the organic layer, which is removed, dried, dissolved and subjected to liquid chromatography mass spectrometry (Waters API Quattro Micro LCMS, Stellenbosch, South Africa) analysis. Isotopically labelled methionine- D_3 was added to the samples as an internal standard.

5.3.7.10. *DMS and methional production (SPME fibre and GC analyses)*

The analyses for dimethyl disulfide (DMS) were conducted according to the method of described by Niki, Fujinaga, Watanabe, and Kinoshita (2004) and Mestdagh, De Meulenaer, De Clippeleer, Devlieghere, and Huyghebaert (2005). Milk samples (3 mL) were weighed and sealed in a 10 mL vial. A Supelco Stableflex polyacrylate

solid phase micro-extraction fibre (SPME) was used to extract the sample at 60 °C for 10 min prior to GCMS analyses. The SPME fibre was manually transferred to headspace vials and the headspace was analysed using gas chromatography mass spectrometry (GCMS). Splitless injections were performed with the injector set at 240 °C (initial injector temperature was set at 40 °C). Helium was used as carrier gas at a flow of 1.50 mL.min⁻¹ through an Agilent 6890 N GC with CTC CombiPAL Autosampler and Agilent 5975B MS. Separation was performed on a DB-FFAP Capillary column (60 m, 0.25 mm ID, 0.5 µm film thickness). Peak identification and validation was done using standard solutions of DMS and methional (100 µg.mL⁻¹) acquired from Sigma-Aldrich, SA.

5.3.7.11. *Urea-PAGE and non-denaturing-PAGE analysis*

Non-denaturing PAGE gels were performed using the method of Andrews (1983). Urea-PAGE gels were performed according to the method of Shalabi and Fox (1987).

5.3.7.12. *Enzyme analysis*

Plasmin and plasminogen activity was determined according to the modified method of Politus, Barbano, and Gorewit (1992). The Storch test was performed on milk samples to determine if the enzyme peroxidase was present or absent in milk after treatment and the Aschaffenburg and Mullen test was used for Milk Alkaline Phosphatase (MAP) enzyme activity (Roberts, 2003).

5.3.8. Sensory Analysis

Sensory evaluations were performed by trained panellists in accordance with the guidelines of the American Dairy Science Association (ADSA) as described by Bodyfelt, Tobias, & Trout (1988). Descriptive sensory analysis was performed on the different treatments of milk. An unstructured 100-mm line scale was used to analyse the sensory characteristics. The panellists were selected and trained in accordance with the generic descriptive analysis technique (Lawless & Heymann, 1995).

5.3.9. Statistical analysis

ANOVA were performed on the data using SAS version 8.12 and ProphetStat was used for median polishing (MP). On the milk data one-way analyses of variance (ANOVA) was performed, using all data from triplicate analyses of three trials compared with the control (untreated) sample in the case of milk. In the instance where the use of ANOVA and t-test analyses was deemed not to be suitable, due non-adherence to certain criteria, a robust statistical method, MP, developed by Tukey, was used (Hoaglin, Mosteller & Tukey, 1983).

Student's t-least significant difference (LSD) was calculated at the 5% significance level to compare treatment means.

5.4. Results and discussion

5.4.1. Effect of thermal and UV treatment on raw milk micro flora

The untreated (raw) full cream milk (RM) was of good quality: RM passed the alcohol test (72% ethanol v/v) and the clot-on-boiling test, and tested negative for antibiotic residues (Kundrat test). Furthermore the somatic cell count of the milk also conformed to Regulation R1555, and results on average were below 300000 somatic cells per millilitre of milk tested (Foss Somatic Cell Counter, Elsenburg, SA). The average log (cfu.mL⁻¹) microbial counts of the untreated (raw) full cream milk was as follows: aerobic plate count (APC), 5.41; coliform count 1.80; aerobic mesophilic spores (AMS), 0.95; anaerobic mesophilic spores (ANMS), 1.57. No counts were detected for *E.coli*, aerobic thermophilic spores (ATS) and anaerobic thermophilic spores (ANTS). The log microbial (cfu.mL⁻¹) counts before and after the respective treatments are given in Table 5.1. The efficacy of the three different treatments applied was similar for that of coliform bacteria, AMS and the ANMS as no counts were detected after the treatments.

Table 5.1: The comparative log microbial counts of full cream raw milk before (RM) and after the respective treatments (P, UV and UVP).

Sample	APC	Coli	<i>E.coli</i>	AMS ⁶	ANMS ⁷	ATS ⁸	ANTS ⁹
RM ¹	5.412	1.803	ND ⁵	0.952	1.579	ND ⁵	ND ⁵
P ²	1.792	ND ⁵	ND ⁵	ND ⁵	ND ⁵	ND ⁵	ND ⁵
UV ³	1.568	ND ⁵	ND ⁵	ND ⁵	ND ⁵	ND ⁵	ND ⁵
UVP ⁴	0.778	ND ⁵	ND ⁵	ND ⁵	ND ⁵	ND ⁵	ND ⁵

¹RM – Raw untreated milk

²P – Treatment 1: Pasteurised Milk

³UV – Treatment 2: UV treated Milk

⁴UVP – Treatment 3: UV and Pasteurised Milk

⁵ND – Not Detected; < 10 cfu.ml⁻¹

⁶AMS – Aerobic Mesophilic Spores

⁷ANMS – Anaerobic Mesophilic Spores

⁸ATS – Aerobic Thermophilic Spores

⁹ANTS – Anaerobic Thermophilic Spores

5.4.2. Effect of thermal and UV treatments on bovine milk composition

5.4.2.1. Macro and micro components

The results of the analysis of chemical composition of milk following the P, UV and UVP treatments are summarised in Table 5.2 and indicated non-significant differences on most of the macro and micro-components of treated full cream milk. The differences between the triplicate samples analysed were not significant ($p > 0.05$ data not shown). According to the statistical analysis (MP) the residual values (RV) calculated between treatments for the RM, P and UV samples were 0, hence indicating no difference between the treatments, however the UVP treatment had a residual value of -0.0067 , hence indicating some differences in some of the constituents measured. The main differences established through the validation of the error values (EV) were that of cholesterol, calcium and sodium on all three treatments. For calcium and sodium the EV calculated with MP were less than that of the standard deviation (STDEV) on the samples measured, thus indicating that the values fall within the expected analytical standard error values measured and were therefore regarded as not significant.

Table 5.2: The chemical composition of full cream milk before (RM) and after the respective treatments (P, UV and UVP).

Nutrient	RM ¹	STDEV	P ²	STDEV	UV ³	STDEV	UVP ⁴	STDEV
Fat (g/100g)	3.60	0.03	3.60	0.02	3.60	0.02	3.55	0.03
<i>Saturated Fat (g/100g)</i>	<i>2.56</i>	<i>0.34</i>	<i>2.58</i>	<i>0.35</i>	<i>2.49</i>	<i>0.20</i>	<i>2.57</i>	<i>0.29</i>
<i>Monounsaturated Fat (g/100g)</i>	<i>0.79</i>	<i>0.10</i>	<i>0.79</i>	<i>0.11</i>	<i>0.82</i>	<i>0.07</i>	<i>0.77</i>	<i>0.07</i>
<i>Polyunsaturated Fat (g/100g)</i>	<i>0.01</i>	<i>0.01</i>	<i>0.08</i>	<i>0.01</i>	<i>0.09</i>	<i>0.01</i>	<i>0.09</i>	<i>0.01</i>
<i>Trans Fatty Acids (g/100g)</i>	<i>0.11</i>	<i>0.01</i>	<i>0.13</i>	<i>0.02</i>	<i>0.14</i>	<i>0.01</i>	<i>0.12</i>	<i>0.02</i>
<i>Miscellaneous (g/100g)</i>	<i>0.05</i>	<i>0.01</i>	<i>0.14</i>	<i>0.01</i>	<i>0.05</i>	<i>0.01</i>	<i>0.05</i>	<i>0.02</i>
Protein (g/100g)	3.30	0.03	3.40	0.02	3.40	0.02	3.40	0.03
Total Carbohydrate (g/100g)	4.70	0.14	4.60	0.17	4.60	0.09	4.60	0.07
<i>Sugars (g/100g)</i>	<i>4.50</i>	<i>0.10</i>	<i>4.50</i>	<i>0.20</i>	<i>4.55</i>	<i>0.20</i>	<i>4.49</i>	<i>0.19</i>
Cholesterol (mg/100g)	12.00	1.12	11.00	1.03	7.70	0.89	9.80	0.94
Calcium (mg/100g)	130.00	1.30	136.00	1.21	136.00	3.22	141.00	1.42
Sodium (mg/100g)	37.40	3.00	39.90	3.21	39.70	2.99	40.30	2.63
Cholecalciferol (Vit. D ₃) (µg/100g)	< 5.00	0.10	< 5.00	0.10	< 5.00	0.10	< 5.00	0.10
Riboflavin (Vit. B ₂) (mg/100g)	0.32	0.05	0.22	0.08	0.31	0.07	0.21	0.08
Vitamin B12 (µg/100g)	0.46	0.03	0.40	0.03	0.48	0.05	0.40	0.04
Biotin (µg/100g)	1.88	0.32	1.95	0.22	1.95	0.25	1.90	0.42
Pantothenic Acid (mg/100g)	0.33	0.04	0.33	0.03	0.28	0.09	0.34	0.08
Ash (g/100g)	0.80	0.10	0.70	0.03	0.60	0.05	0.70	0.07
Moisture (g/100g)	87.60	2.34	87.70	2.42	87.80	2.45	87.75	2.53
Total Solids (g/100g)	12.40	0.53	12.30	0.56	12.20	0.62	12.25	0.82

¹ RM – Raw untreated milk

² P – Treatment 1: Pasteurised Milk

³ UV – Treatment 2: UV treated Milk

⁴ UVP – Treatment 3: UV treated and Pasteurised Milk

5.4.2.2. Riboflavin and Vitamin B₁₂

From the data shown in Table 5.2 it is evident that the heat was the major factor affecting reduction of riboflavin (average ~31%) and vitamin B₁₂ (average ~13%) when comparing the P and UVP treatments with the RM and UV treatment, although the effect measured was not as significant according to the MP model as that of calcium, sodium and cholesterol (i.e. RV value above upper hinge but within inner fence). No decrease in riboflavin and vitamin B₁₂ content was found after raw milk treatment in the UV turbulent flow system. This finding is consistent with reports that Riboflavin and vitamin B₁₂ are reduced by heat treatment (Walstra & Jenness, 1984). The reduction of riboflavin in this instance was only evident in the pasteurised and UV-heat treated samples and these findings are consistent with reports that under

alkaline conditions (such as milk) riboflavin is labile to thermal decomposition, although it is known that riboflavin is also susceptible for decomposition with exposure to light (in particular wavelengths in the range of 415–455 nm). In a study of Webster, Duncan, Marcy, and O'Keefe (2009), different visible and UV riboflavin excitation wavelengths were investigated to try and determine the excitation wavelength or wavelength range responsible for producing light oxidised flavours in milk. It was concluded that blocking of all visible and UV excitation wavelengths in milk packaging was the most successful in reducing light oxidised flavour and that the blocking of single visible and all UV excitation wavelength alone did not completely reduce off-flavour development. In our study no significant difference between the UV treated samples was noted when compared to the RM samples indicating that short exposure time to UV light, UV intensity and wave-lengths of 254 nm do not seem to affect riboflavin decomposition in this instance.

Cow's milk is a source of riboflavin and vitamin B₁₂, on average contributing approximately ~25% and ~40% respectively of the recommended dietary allowance (RDA) for persons older than 10 years. Riboflavin occurs usually in free and a protein bound form in cow's milk, with the free form being the most abundant (~61%). Riboflavin acts as an intermediary in the transfer of electrons in various essential oxidation–reduction reactions and is also important for the production of energy via the respiratory chain. Flavoproteins (riboflavin dependant enzymes) are essential for the conversion of pyridoxine (vitamin B₆) and folic acid into the coenzyme forms, and for the transformation of tryptophan to niacin. Vitamin B₁₂, only produced by microorganisms, is important in the human diet as it is exclusively obtained from animal-derived foods. Vitamin B₁₂ is important for the formation of blood corpuscles, nerves sheaths and various proteins are essential for growth, is important in carbohydrate and lipid metabolism, DNA synthesis and regeneration and formation of red blood cells.

5.4.2.3. *Lipids*

No significant difference was recorded when comparing the percentages fat, saturated fat, monounsaturated fat, polyunsaturated fat and trans fatty acids as determined by GC and given in Table 5.2. However, in the case of the miscellaneous fat constituents the concentration of sample P (0.130 g per 100 g) was approximately 2.5-fold higher when compared to that of the RM (0.05 g per 100 g), UV (0.054 g per 100 g) and UVP (0.045 g per 100 g) treatments. However, this value is calculated and not directly measured as the difference between the total fat percentage and the sum of the saturated fat, monounsaturated fat, polyunsaturated fat and trans fatty acid components, which was directly measured by CG method. Therefore any experimental error would be summated in the calculated miscellaneous fat component concentration. The contribution of the miscellaneous fat components is minor and usually contributes less than 2% of the total fat fraction, as was the case in this study. The fraction is made up of phospholipids, sterols, and carotenoids and could also contain trace amounts of fat-soluble vitamins and flavour compounds.

5.4.2.4. *Cholesterol*

The effects of P, UV and UVP treatments applied to RM on cholesterol indicated differences in concentrations after the treatments (EV: RM = 1.60, P = 0.60, UV = - 2.70 and UVP = - 0.60). As seen from Table 5.2 the most noticeable change in cholesterol was the values achieved following treatments that included UV, i.e. the UV and UVP samples, which indicated a 36% and 18% reduction in cholesterol respectively when compared with the RM.

Cholesterol, an unsaturated lipid, is susceptible to oxidation under a variety of conditions. Various authors have reported the formation of cholesterol oxidation products (COPs) in many foods due to the auto-oxidation of cholesterol in presence of light, heat and pro-oxidants (Kumar & Singhal, 1991; Sander, Smith, Addis, & Park, 1989; Smith, 1992). During auto-oxidation hydroperoxides are formed from polyunsaturated fatty acids, which may react with cholesterol causing the removal of

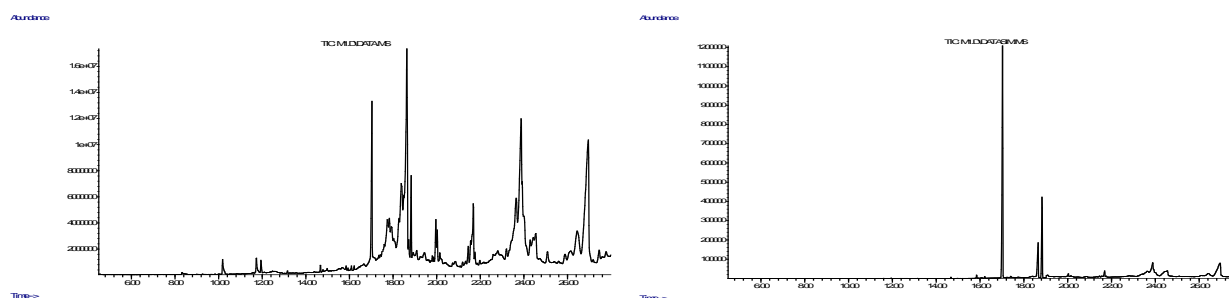
hydrogen from the $\Delta 5-6$ double bond. This introduces a free radical into the cholesterol structure that readily migrates to positions 4 and 7 of the A and B rings of cholesterol, respectively. The latter is the most stable and preferred position and one of the three end products, as a result of oxidation, is 7-ketocholesterol. The hydroperoxides could ultimately lead to the formation of compounds resulting in off-flavours, such as ketones, aldehydes, alcohols, hydrocarbons, acids and epoxides (Menéndez-Carreño, Ansorena, & Astiasarán, 2008). 7-Ketocholesterol is the major cholesterol oxide formed, and a good correlation between thiobarbituric acid reactive substances (TBARs) and COPs concentration was observed in a study of Angulo, Romera, Ramirez, and Gil (1997). To verify the possible oxidation of cholesterol caused by UV and/or heat treatment of the milk, 7-ketocholesterol was measured as an indicator of COPs. However, all the values obtained for 7-ketocholesterol were less than $10 \mu\text{g.g}^{-1}$ fat and showed little variance (CV 3.7%). Although these values are lower than the reported values of Kumar and Singhal (1991) it is important to note that the heat treatments allowed in their study was higher (HTST: 85°C for 16 s and UHT: 140°C for 4 s) than that applied for the manufacturing of the Cheddar cheese in this instance (HTST: 73.5°C for 15 s). In Figure 5.1, the full GC-MS scans of the 4 milk samples accompanied by the Single Ion Monitoring (SIM) scans are shown. The molecular masses for the internal standard (5 α -cholestane), siliated cholesterol and 7-ketocholesterol were monitored with single-ion monitoring (SIM).

5.4.2.5. TBARs analysis

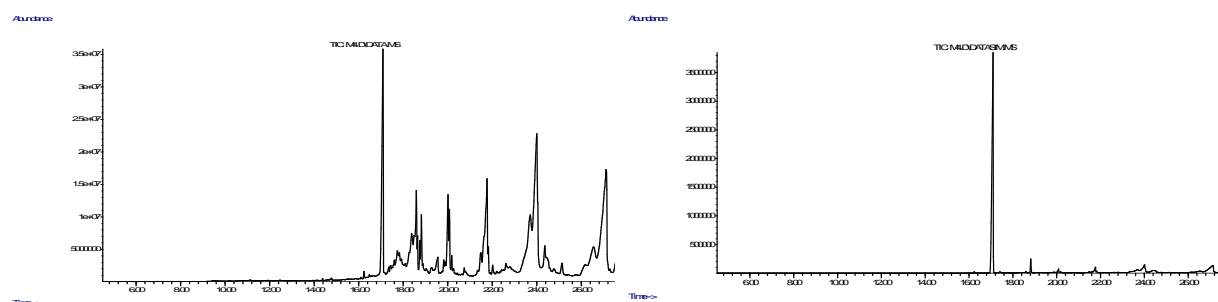
In order to further characterise possible oxidative deterioration, the treated milk was also analysed for thiobarbituric acid reactive substances (TBARs) (Table 5.3). During lipid oxidation, malonaldehyde (MA) is formed as a result of the degradation of polyunsaturated fatty acids. In a study conducted by Matak *et al.* (2005) of UV irradiated goat milk exposed at 16 J.cm^{-2} for 18 s, it was demonstrated that the applied UV dose increased the TBARs values two fold over that of the control samples.

Figure 5.1: GC-MS chromatograms of the 4 milk samples accompanied by the single ion monitoring (SIM) scans are shown. The molecular masses for the internal standard (5 α -cholestane), siliated cholesterol and 7-ketocholesterol were monitored with SIM.

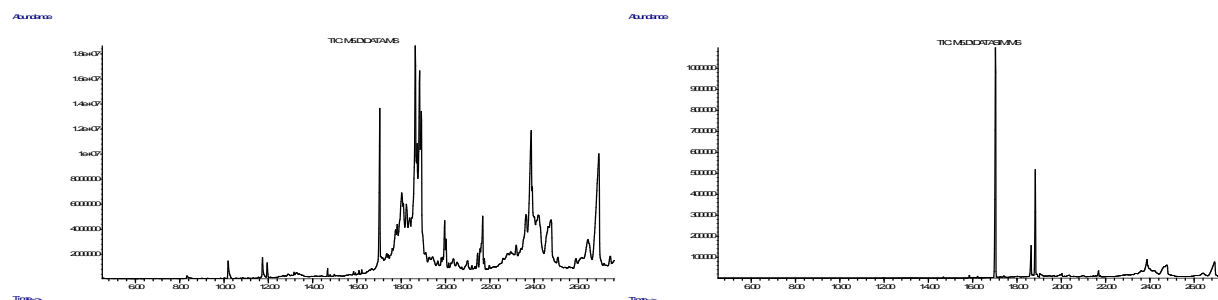
RM - Untreated (Raw) Milk



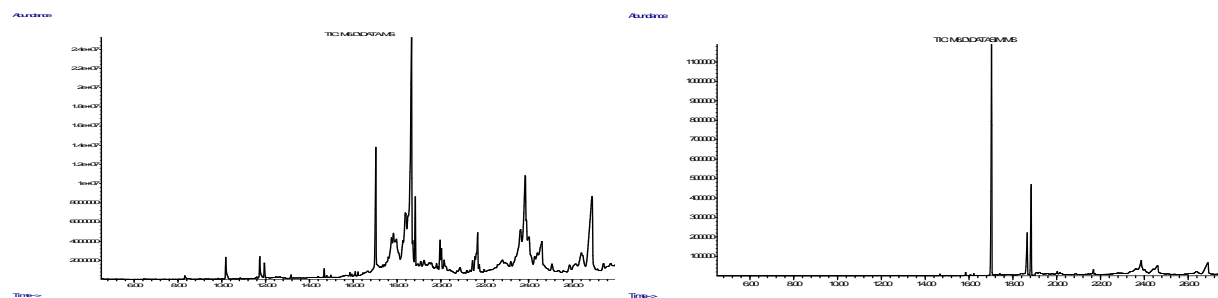
P - Pasteurised Milk



UV - UV Milk



UVP – UV & Pasteurised Milk



The TBARs results achieved for this study indicated no statistical difference ($p > 0.05$) in the absorption values (530–535 nm) of the four milk samples analysed, therefore also confirming the results achieved with the 7-ketocholesterol determination. The TBARs values obtained and expressed as milligrams of MA equivalents per kilogram sample (ppm), are given in Table 5.3. The results achieved is contrary to the report of Van Aardt *et al.* (2001) who found that milk exposed to light for 10 h had significantly greater TBARS values than milk that was protected from light exposure. However, only one UV-C dose was used in our study at a consistent UV-C dose of 430 mJ.cm^{-2} (1045 J.L^{-1}) for a relatively short period of time (less than 25 s as supposed to 10 h).

Table 5.3: TBARs, %FFA and Acid Degree Value values of full cream milk (RM) before and after the respective treatments (P, UV and UVP).

Sample	TBARs value (ppm) ¹	STDEV	% FFA (as % oleic acid) ²	SDTEV	ADV (mEq/100g Fat)	SDTEV
RM	0.220	0.030	0.291	0.034	0.920	0.320
P	0.250	0.018	0.313	0.029	0.897	0.436
UV	0.230	0.025	0.365	0.037	1.000	0.414
UVP	0.255	0.040	0.511	0.044	2.650	0.699

¹TBARs - expressed as milligrams of MA equivalents per kilogram sample (ppm) or as micromoles of MA equivalents per gram of sample

² % FFA – expressed as % free fatty acids as % oleic acid (*m/m*)

³ ADV – expressed as millilitres of 1 N KOH per 100 g of fat

Humans are able to absorb COPs from foods directly into the bloodstream and undesirable implications, as far as human health is concerned, have been reported by various authors (Emanuel, Hassel, Addis, Bergmann, & Zavoral, 1991). Impaired cholesterol biosynthesis, alteration of membrane function, cytotoxicity, and factoring in atherosclerosis are a few of the negative aspects associated with the intake of COPs (Smith & Johnson, 1989). In lieu of the aforementioned it is important to highlight that no significant difference between the treatments as far as the production of COPs were observed as confirmed by the TBARs and 7-ketocholesterol results.

5.4.2.6. Free fatty acid (FFA) analysis

The results of FFA analysis are summarised in Table 5.3. From the results expressed as % FFA as oleic acid, it is evident that there was a significant increase (~75%) of %FFA when comparing the UVP treatment with the P and UV treatments ($p < 0.05$). The %FFA of the RM measured was 0.29%, compared to the respective values of P, UV and UVP of 0.31%, 0.37% and 0.51%. The possible cause could have been the damage to the milk fat globule membrane (MFGM), which accelerated oxidative rancidity, due to excessive pumping of the milk (i.e. double processing through the UV turbulent system and the HTST pasteuriser). This was confirmed by the observation of free-fat (oil-droplets) on the surface of the milk during inoculation of the cheese culture, and also confirms the higher % fat loss in the whey as supposed to the other treatments (results not shown). The ADV results confirmed the results indicating lipolysis when comparing the UVP sample with the RM, P and UV treatments. In general ADV values of $N \ 1.0 \text{ meq}/100 \text{ g}$ in cow's milk usually is indicative of rancid off-flavours present in the milk. A possible cause could be the partial homogenisation of the milk through the UV system before pasteurisation, which increases the risk of lipolysis. However, the same trend would then have been expected to be present in the UV treated sample, therefore the damage to the MFGM seems to be the most likely cause. This finding is also consistent with reports of Matak *et al.* (2005), where an increase in the ADV was observed circulating the milk 16-times through a similar UV device without switching on the UV source, when compared to the initial (control) milk sample. The same methodology was followed by pumping full cream milk through the SP-40 without switching on the UV lights, and the reported increase in %FFA on average recorded was ~50%, thus confirming the observations of Matak *et al.* (2005) (results not shown).

In Table 5.4, the fatty acid profile of the raw full cream milk (RM) is compared with the full cream milk samples after each treatment. The major fatty acids found in milk fat are butanoic (C4:0), hexanoic (C6:0), octanoic (C8:0), decanoic (C10:0), dodecanoic (C12:0), tetradecanoic (C14:0), hexadecanoic (C16:0), octadecanoic (C18:0), cis-9-octadecenoic (C18:1), cis, cis-9,12-octadecadienoic (C18:2), and

9,12,15-octadecatrienoic acids (C18:3). Of these fatty acid fractions approximately 30% of total fatty acids is comprised of the saturated fatty acid, hexadecanoic acid (C16:0), while tetradecanoic (C 14:0) and octadecanoic (C 18:0) fatty acids contributes 10 to 13% of total fatty acids in normal milk (Fox and McSweeney, 2006). For most fatty acids, there were no significant difference between the untreated RM and P, UV and PUV treated full cream milks samples as well as for the total trans fatty acids, total saturated fat, total mono-unsaturated fat and total poly-unsaturated fat fractions also did not reveal any statistical differences when applying the median polishing model. The model indicated that all residual values were near zero, except for C18:1 cis n9c (oleic acid) with a residue of 1.15 in the UV treated sample and C 18:0 (stearic acid) in the RM sample with a residual value of -1.05. From these results it was evident that these 2 parameters tested were statistically significant. Timmons, Weiss, Palmquist, and Harper (2001) reported that as the concentrations of unsaturated fatty acids in milk increase (PUFA C18:2 and C18:3), the milk will become more susceptible to oxidation and production of off-flavours could develop in the case of UV exposure.

5.4.2.7. *Protein oxidation*

The results of the analysis of the milk for DMS, methionine, methionine sulfoxide and methionine sulfone after P, UV and UVP treatments are indicated in Table 5.5 and Figure 5.2. From the results in Table 5.5 it is evident that the DMS concentration in the RM sample (0.070 nM) and P sample (0.072 nM) was higher in comparison with that of the UV treated milk samples, UV (0.038 nM) and UVP (0.064 nM).

Table 5.4: Fatty acid profile % w/w of full cream milk before (RM) and after the respective treatments (P, UV and UVP).

Fatty Acids	RM ¹	STDEV	P ²	STDEV	UV ³	STDEV	UVP ⁴	STDEV
C4:0 (Butyric)	3.60	0.23	3.00	0.32	2.50	0.42	3.30	0.25
C6:0 (Caproic)	2.60	0.21	2.40	0.23	2.00	0.23	2.30	0.31
C8:0 (Caprylic)	1.50	0.08	1.50	0.12	1.20	0.11	1.40	0.10
C10:0 (Capric)	3.40	0.15	3.50	0.19	2.80	0.15	3.40	0.13
C10:1	0.40	0.04	0.40	0.02	0.30	0.04	0.30	0.02
C12:0 (Lauric)	3.90	0.18	3.90	0.19	3.40	0.18	4.00	0.20
C12:1	0.20	0.03	0.20	0.04	0.20	0.03	0.20	0.04
C13:0 (Tridecanoic)	0.10	0.03	0.10	0.02	0.10	0.02	0.10	0.02
C14:0 (Myristic)	11.60	0.45	12.10	0.50	11.20	0.73	12.40	0.45
C14:1 (Myristoleic)	1.00	0.06	0.70	0.08	0.70	0.04	0.70	0.05
C15:0 (Pentadecanoic)	1.50	0.07	1.30	0.09	1.40	0.12	1.30	0.07
C15:1 (<i>cis</i> -10-Pentadecanoic)	0.30	0.02	0.30	0.03	0.30	0.03	0.30	0.03
C16:0 (Palmitic)	29.10	0.28	29.70	0.25	29.50	0.27	30.30	0.30
C16:1 (Palmitoleic)	1.60	0.08	1.60	0.12	1.60	0.13	1.60	0.04
C17:0 (Heptadecanoic iso)	0.80	0.07	0.60	0.03	0.80	0.04	0.60	0.08
C17:0 (Heptadecanoic anteiso)	0.60	0.05	0.60	0.04	0.60	0.07	0.60	0.09
C17:0 (Heptadecanoic)	0.70	0.03	0.10	0.01	0.30	0.05	0.10	0.03
C17:1 (<i>cis</i> -10-Heptadecanoic)	0.20	0.04	0.20	0.04	0.20	0.03	0.20	0.06
C18:0 (Stearic)	11.00	0.45	12.00	0.53	12.70	0.22	12.10	0.45
C18:1 <i>cis</i> n9c (Oleic acid)	17.50	0.48	17.90	0.52	19.10	0.58	18.00	0.29
C18:1 <i>trans</i> n9t (Elaidic)	0.60	0.03	0.70	0.03	0.50	0.03	0.60	0.05
C18:2 n6c (Linoleic acid)	2.20	0.15	1.90	0.17	2.10	0.15	2.00	0.14
C18:2 Conjugated CLA	0.10	0.02	0.10	0.02	0.10	0.01	0.10	0.03
C18:3 3n3 (alpha-Linolenic acid)	0.50	0.04	0.40	0.03	0.50	0.04	0.40	0.04
C20:0 (Arachidic)	0.50	0.02	0.70	0.04	0.60	0.02	0.50	0.02
C:22:0 (Behenic)	0.10	0.02	0.10	0.01	0.10	0.01	0.10	0.01
Total Trans Fatty Acids	3.10	0.17	3.60	0.35	3.90	0.15	3.30	0.18
Total Saturated Fat	71.00	1.58	71.60	1.77	69.10	1.63	72.30	1.98
Total Mono-Unsaturated Fat	21.80	1.10	21.90	0.84	22.90	0.98	21.80	0.77
Total Poly-Unsaturated Fat	2.70	0.17	2.30	0.39	2.50	0.32	2.40	0.30
Miscellaneous	1.40	0.10	0.60	0.13	1.60	0.14	0.20	0.07

¹RM – Raw untreated milk

²P – Treatment 1: Pasteurised Milk

³UV – Treatment 2: UV treated Milk

⁴UVP – Treatment 3: UV treated and Pasteurised Milk

Table 5.5: The concentration of methional, dimethyl disulfide (DMS), methionine, methionine sulfoxide, and methionine sulfone of full cream milk before (RM) and after the respective treatments (P, UV and UVP).

Milk Sample	Methional [mM]	STDEV	DMS [nM]	STDEV	Met ($\mu\text{g.mL}^{-1}$)	STDEV	Met Sulfoxide ($\mu\text{g.mL}^{-1}$)	STDEV	Met Sulfone ($\mu\text{g.mL}^{-1}$)	STDEV
RM	< 0.010	0.0005	0.0700	0.004	36.300	1.815	10.630	0.532	0.580	0.043
P	< 0.010	0.0005	0.0720	0.005	39.280	1.964	9.630	0.600	0.625	0.033
UV	< 0.010	0.0005	0.0380	0.005	40.920	2.140	11.020	0.500	0.970	0.054
UVP	< 0.010	0.0005	0.0639	0.005	39.000	1.873	9.460	0.473	1.090	0.055

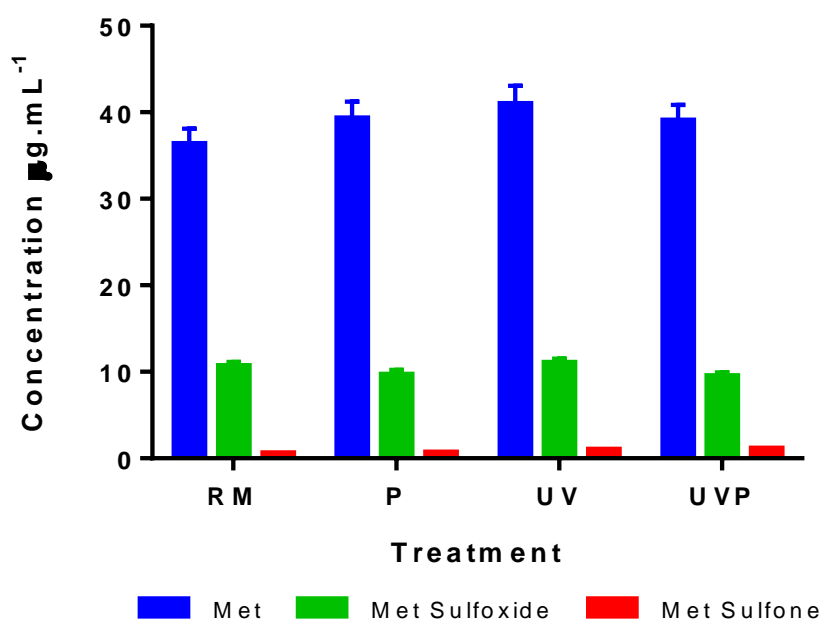


Figure 5.2: Differences in dimethyl disulfide (DMS), methionine, methionine sulfoxide, and methionine sulfone of full cream milk before (RM) and after the respective treatments (P, UV and UVP).

The role of protein oxidation and the subsequent production of off flavours, such as a burnt or sunlight flavour, has been regarded as less important than lipid oxidation in the past. However, various studies have shown that protein oxidation, and specifically photo-oxidation of proteins, indeed significantly contributes to the deterioration of

milk quality and the production of similar off flavours (Dimick, 1976; Trine, Dalsgaard, Nielsen, & Larsen, 2007). Light-induced flavours formed by the Type II reaction of riboflavin are attributed to the degradation products of the amino acid methionine. Methionine undergoes Strecker degradation in the presence of singlet-oxygen to form methional, dimethyl disulfide, and many other aldehyde and sulfur compounds (Allen & Parks, 1975; Forss, 1979; Korycka-Dahl & Richardson, 1978; White & White, 1995). Initially it was believed that methional, responsible for a burnt feather and/or cabbage-like flavour, was the main component formed following photo-oxidation. However, recent studies indicated that methional is converted to methanethiol through decarboxylation is also converted to dimethyl disulfide (DMS) and dimethyl trisulfide (Milo & Reineccius, 1997). All the samples in this study tested < 1 ppb for methional (sensory flavour thresholds is 0.05–10 ppb) (Fors, 1988; Patton, Forss, and Day, 1956; Schutte, 1974). Trine *et al.* (2007) reported that photo-oxidation in the presence of the photosensitizer riboflavin resulted in changes in the primary structures of all the milk protein preparations studied: α -, β -, and κ -casein as well as the globular proteins α -lactalbumin and β -lactoglobulin as well as lactoferrin. Studies of Hui (1993) and Jung, Yoon, Lee, and Min (1998) indicated that methionine reacts with singlet oxygen, produced by riboflavin, to yield DMS, methyl sulfide, methionine sulfoxide, methionine sulfone and other sulfur-containing compounds (Lee & Min, 2009). Byproducts of this reaction are ammonia and carbon dioxide and these compounds give milk a similar odour to that of lipid oxidised milk (Hui, 1993). Studies have indicated that DMS, an organosulfur compound, could be readily transferred from the rumen to the milk, producing a characteristic disagreeable odour associated with cabbage-like smells and feed, “cowy” or “barn”-like off-flavours (Dunham, Ward, Bassette, & Reddy, 1968; Shipe *et al.*, 1962). Low olfactory threshold levels are prominent in individuals and can vary between 0.06 ppb in water to 19 ppb in milk (Patton *et al.*, 1956; Schutte, 1974). In this study the highest concentration of DMS was detected in the RM and P at 0.004 ppb, with no increase in the DMS concentration following the UV and UVP treatments.

The differences in methionine and methionine sulfoxide were not significant ($p > 0.05$), however, there was a significant increase in the concentration of methionine sulfone in all treated milk samples, P, UV and UVP, following the respective treatments. Increases in the levels of methionine sulfone were 7.8% (P), 67% (UV)

and 88% (UVP) when compared with the RM sample. The highest concentration of methionine sulfone was detected in the milk treated with UV. Methionine sulfone, unlike methionine sulfoxide, is a product of complete oxidation of methionine and is an indicator of oxidative damage to proteins. This finding is also consistent with the reports of Allen and Parks (1975) that reported that as the sunlight flavour in milk developed, riboflavin, which acts as a photosensitizer, was destroyed. In their findings the destruction of riboflavin in milk was described as a 1st order reaction in relation to the exposure time to light (Allen & Parks, 1975). These findings are also consistent with the reduction of riboflavin noted in this study, when comparing the RM with the treated milk samples. However, no significant decrease in methionine between the different milk samples was observed, as would have been expected, and the levels of methionine sulfone determined was much lower than that of methionine and the methionine sulfone. Therefore, the effect of photo-oxidation and the formation of methionine sulfone and the organoleptic impact and threshold values (detection limits), should be further investigated.

5.4.2.8. *Proteolysis and enzyme activity*

To determine the level of proteolysis of the RM and treated milk samples, the TNBS method and absorption of the milk serum at 280 nm were utilised. It was important to confirm if differences occurred between the RM and milk treatments as the milk was used for further processing and the production of Cheddar cheese (part 2 of the study). Enzymes from several sources contribute to proteolysis and the subsequent development of cheese texture and flavour during ripening. These enzymes originate from the milk (principally plasmin), the coagulant (rennet), starter, secondary starter and non-starter microorganisms.

The TNBS method confirmed that there were no significant differences ($p > 0.05$) in the α -amino acids in milk treated with P, UV and UVP. As shown in Fig. 3, the α -amino acids in milk were within the range of 39.2–43.1 mg.mL⁻¹ for each treatment. As indicated in Fig. 5.4 there was also no significant differences detected ($p > 0.05$) in the absorption values measured at 280 nm of milk between treatments, hence indicating no statistical difference in the aromatic amino acid side chains of short peptides of hydrolysed casein molecules measured. Both the non-denaturing and Urea

PAGE gels, as shown in Figures 5.5 and 5.6, confirmed these observations and also did not show any differences between the protein profiles of the RM when compared with the treated milk samples.

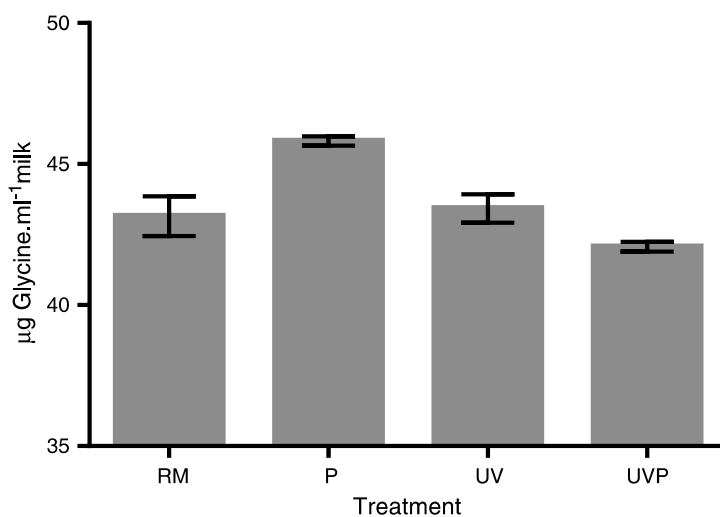


Figure 5.3: The α -amino acid content of full cream milk before (RM) and after the respective treatments (P, UV and UVP) using the TNBS method.

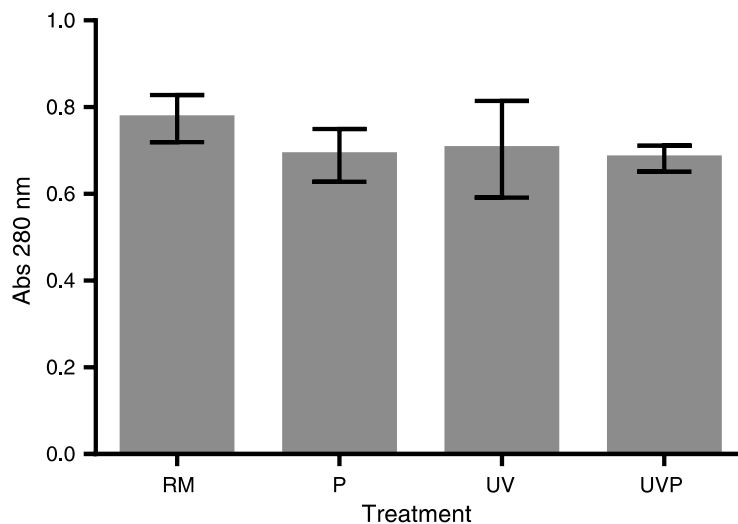


Figure 5.4: The absorption values at 280nm of full cream milk before (RM) and after the respective treatments (P, UV and UVP).

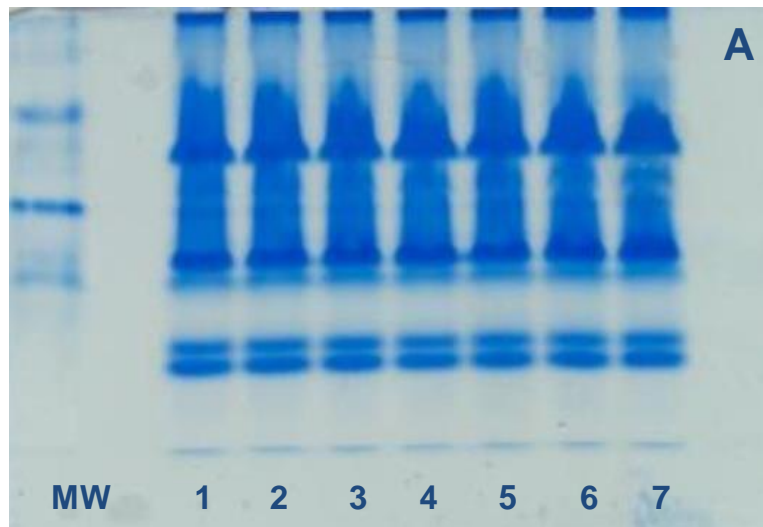


Figure 5.5: Non-denaturing PAGE gel of the milk samples: MW, Molecular Marker; Lane 1, RM; Lane 2, RM; Lane 3, RM; Lane 4, P; Lane 5, UV; Lane 6, UVP and Lane 7, P.

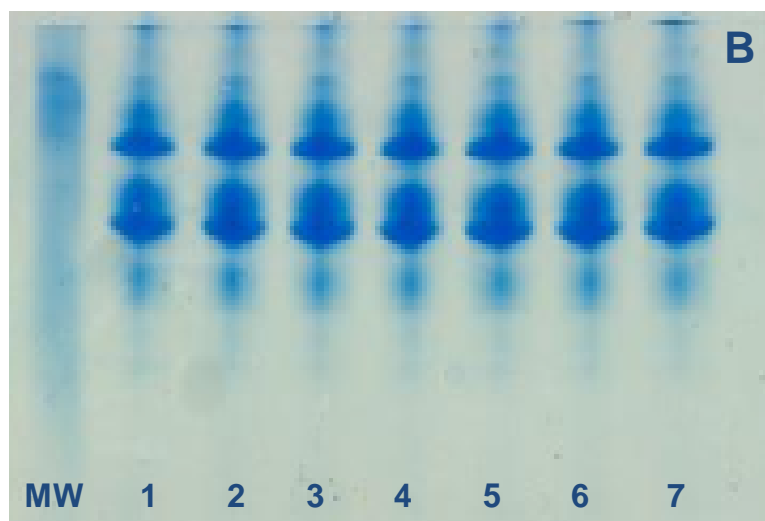


Figure 5.6: Urea PAGE gel of the milk samples: MW, Molecular Marker; Lane 1, RM; Lane 2, RM; Lane 3, RM; Lane 4, P; Lane 5, UV; Lane 6, UVP and Lane 7, P.

Qualitative tests (shown in Table 5.6) indicated no differences between the activities of phosphatase and peroxidase in RM and UV treated milk. In the instance when heat treatments were applied (P and UVP) no residual phosphatase activity was detected, which is used in the dairy industry as an indicator test for sufficient . Therefore in the instance where UV-treatment of milk is considered as a standalone technology, the current phosphatase test used to qualify sufficient milk will not be suitable to validate and qualify effective UV-treatment and UV-C dose delivered to the milk. However it should be noted that the phosphatase test serves only as an indicator of sufficient heat treatment as reactivation of the phosphatase enzyme could occur post- with the heating of the milk and/or contact with the skin. Therefore caution should be applied to see the test as absolute; nevertheless as a rapid qualitative it does have value within a commercial environment as screening a test to assess effective heat transfer during . Ultimately the results of microbiological testing serve as the final release criteria for commercial pasteurised milk, and therefore the same methodology should be followed when processing milk with UV. It remains of interest though to develop a rapid screening test, such as the phosphatase test in pasteurised milk, to qualify whether the UV treatment and UV-C dose was effectively applied with the treatment of milk. The development of rapid, automated analytical techniques to assess microbiological quality of final products produced could in future also be considered a for both UV-treatment and of milk.

Milk alkaline protease (MAP), commonly referred to as plasmin, is one of the major enzymes in milk. Under certain physiological conditions, such as bacterial infection, the inactive precursor zymogen, plasminogen (PG), is converted into the active form, plasmin (PL), by self-activation (autolysis) or by limited proteolysis by another protease (Lu & Nielsen, 1993a,b; Richardson, 1983; Zachos, Politus, Gorewit, & Babano, 1992). PL readily hydrolyses the α s2- and β -casein at the same rate to form shorter peptides referred to as γ -casein and the proteose peptones, and plays an important role in proteolysis in milk and cheese (Andrews, 1983; Fox & McSweeney, 2003). The activity of plasmin and plasminogen were quantitatively determined on the RM and treated milk samples as indicated in Fig. 7. The results indicated no statistical difference ($p > 0.05$) between the PG activity measured between the RM

and the treatments. There seemed to be some differences in the PL (or activated form of the enzyme) measured between the RM, P and UV, UVP treatments. However, the STDEV of the tests were high in comparison to that of the results of PG, and detection levels were low. The ratio of PL to PG is an important indicator of PG conversion; the ratios measured in this instance did not indicate a significant difference ($p > 0.05$) in plasminogen conversion except for the UV and UVP treatments. This should be further investigated as there are various factors influencing the conversion of PG to PL such as plasminogen activators, plasmin inhibitors and plasminogen activator inhibitors that could have been affected by UV treatment (Fox & Morrissey, 1981; Kitchen, 1985). However, this seems unlikely as the activity of PG did not significantly vary.

Table 5.6: Qualitative tests of milk samples for phosphatase and peroxidase activity of full cream milk before (RM) and after the respective treatments (P, UV and UVP).

Milk Sample	Phosphatase¹	Peroxidase²
RM	+++	+++
P	-	+++
UV	+++	+++
UVP	-	+++

¹ Phosphatase test according to the methodology of Aschaffenburg and Mullen test for the phosphatase enzyme activity as described in the Regulations relating to Milk and Dairy Products, Published under Government Notice No. R. 1555 of 21 November 1997).

² Peroxidase test according to the Storch method performed on milk samples to determine if the enzyme peroxidase is present or absent in milk, according to the national standard method certified by the National Public Health Service of Wales

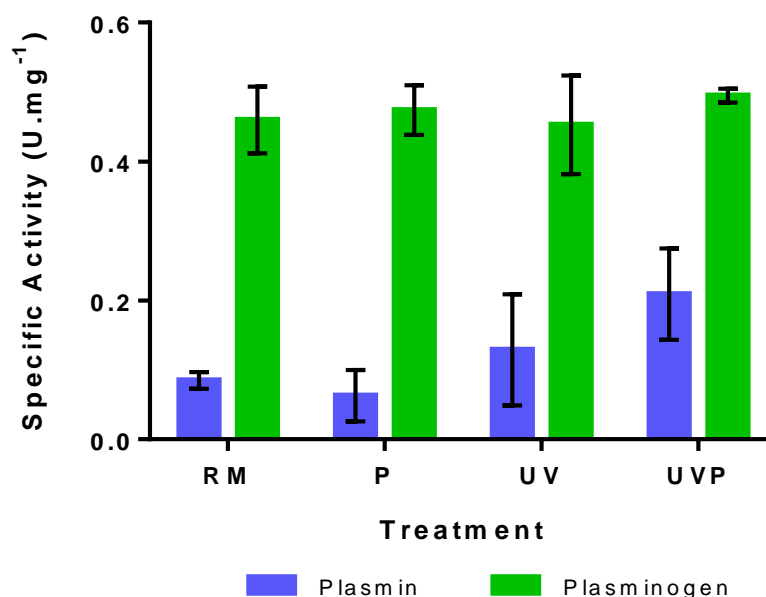


Figure 5.7: The plasmin and plasminogen concentration of full cream milk before (RM) and after the respective treatments (P, UV and UVP).

5.4.3. Effect of heat pasteurisation and UV treatments on sensory attributes of milk

The results of the sensory analysis are summarized in Table 5.7. Sensory analysis was only conducted on the P, UV and UVP samples, excluding the RM samples. In this instance pasteurised milk (P) was considered as the control. The results indicated that the UV milk was perceived as having a definite and noticeable creamy milk flavour and aroma, but lower than that of the P and UVP samples ($p < 0.05$). No differences in sour milk aroma or flavour were detected in any of the samples measured ($p > 0.05$). As an indication of possible oxidation two main descriptors were used for the sensory analysis, namely “tallow” and “cardboard”. DMS and methional derived from oxidation of sulfur containing amino acids are reported to be responsible for the “tallowy or sunlight” flavour whereas “cardboard-like or metallic” flavour usually develops in milk with prolonged duration of light exposure and is as a result of secondary lipid oxidation products such as hexanal, pentanal, ketones, alcohols, and hydrocarbons (Lee & Min, 2009).

For the 'tallowy', or sunlight descriptor, the UV treatment rated higher than the P and UVP samples in terms of aroma and flavour. Mean values of less than five can be regarded as not noticeable. No cardboard aroma or flavour could be detected in the control (P) sample, and the UVP sample showed higher values for this attributes when compared to UV ($p < 0.05$). However, the cardboard note was less prominent than that of the tallowy aroma and flavour for all treatments.

The results of the sensory analysis correlated with the findings of Rossitto *et al.* (2012) who tested the microbial and sensory characteristics of milk treated by the pilot scale turbulent UV system with 4 turbulators using recirculation. In their study sensory defects were noted after treating milk with UV at dosages levels of 880 and 1760 J.L⁻¹, with the severity of the defects increasing as the UV dosage levels increased. The most noticeable off-flavours they found were attributed to lipid oxidation and an increase in TBAR's, and described as 'burnt', 'off', 'strong' and 'stale'. However, they concluded that these observations were not quantitative and that some of the panellists in the triangle test could not taste a difference between the control and UV treated samples. Their concluding remark was that further research need to be conducted, specifically addressing microbial efficacy and sensory attributes in milk treated in the range of 880–1760 J.L⁻¹, to establish optimal germicidal efficacy and reducing possible sensory defects. However, the observed sensory defects could be attributed to the overexposure of milk portions to the UV photons due to recirculation character of UV treatment in the pilot SP 4 unit. The advantage of the commercial scale turbulent flow SP 40 is that the milk was exposed to the identical UV dose flowing through 40 single turbulators and thus achieving better mixing conditions and more uniform UV treatment of raw milk despite of its opaque UV characteristics.

Table 5.7: Mean sensory scores for the treated milks on the following descriptors: (1) creamy milk aroma and flavour, (2) sour milk aroma and flavour, (3) tallowy milk aroma and flavour, (4) cardboary milk aroma and flavour, (5) sweetness and (6) mouthfeel.

Treatment	Mean values of sensory scores									
	Creamy milk aroma	Sour milk aroma	Oxidised aroma: Tallowy	Oxidised aroma: Cardboard	Creamy milk flavour	Sour milk flavour	Oxidised flavour: Tallowy	Oxidised flavour: Cardboard	Sweetness	Mouth feel
P	50.00 ^a	0.00 ^a	0.50 ^d	0.00 ^d	50.00 ^a	0.00 ^a	1.00 ^d	1.00 ^d	50.00 ^a	47.00 ^a
UV	34.5 ^c	0.00 ^a	9.90 ^c	6.75 ^c	40.33 ^b	0.00 ^a	9.90 ^c	5.75 ^c	38.90 ^{bc}	39.25 ^b
UVP	43.34 ^b	0.00 ^a	4.90 ^d	2.05 ^d	42.18 ^a	0.00 ^a	4.00 ^d	2.11 ^d	47.05 ^a	47.00 ^a
LSD	3.1	0	5.25	2.65	3.15	0	4.91	5.11	3.00	3.00

LSD, Least Significant Difference at P = 0.05

^{a-d} Values in the same column with different superscripts are significantly different, $p < 0.05$.

5.5. Conclusion

Three different treatments P, UV and UVP were applied to raw milk to determine the comparative microbial efficacy, chemical, biochemical and sensory character of the resulting processed milks produced. Ultraviolet light turbulent flow system (SP-40 unit) was validated as a novel processing technology to investigate the feasibility of using it as an alternative processing technology to traditional thermal pasteurisation as a stand-alone technology or as an adjunct to thermal pasteurisation when processing milk for the manufacture of cheese on a commercial scale.

In this study, the microbial results indicated that the UV technology achieved the same microbial efficacy on the parameters measured when comparing UV to traditional heat pasteurisation. The use of UV in combination with thermal pasteurisation achieved the highest reduction on APC (4.3-log), as would be expected, however, it is important to note that the milk used was of good quality and conformed to parameters specified for milk for further processing as per legislation in South Africa. Future investigation should include challenge testing, specifically relating to pathogens, to determine the germicidal efficacy when using UV technology as an alternative or adjunct to pasteurisation. This study should include the identification of

the most UV resistant pathogen, such as *Coxiella burnetii* in the instance of thermal pasteurisation and *Listeria monocytogenes* for cheese milk treatment.

As far as the macro- and micronutrient composition of milk is compared some different effects were noted between the treatments. The most noticeable change was a 35% reduction in cholesterol with UV treatment and 18% reduction in cholesterol with the UVP treatment, which indicates that UV does reduce the cholesterol and result in the possible conversion of cholesterol to COP's. In this instance 7-ketocholesterol, measured less than 10 $\mu\text{g}\cdot\text{g}^{-1}$ fat in all treatments, which was confirmed by the measurement of the thiobarbituric acid (TBA) reactive substances. The reduction of cholesterol should be further investigated and the exact metabolites (COP's) formed should be identified. As would be expected the thermal treatments (P and UVP) reduced riboflavin and vitamin B₁₂ in milk. Industry and consumers in general accept the minor changes to such components following thermal treatment, as it is necessary to provide a safer final product with an increased shelf life.

No differences in protein oxidation products between tested treatments and RM on methional, methionine, methionine sulfoxide and dimethyl disulfide (DMS) were measured in this study. In fact, the UV sample had the lowest concentration of DMS when compared to other treatments (0.04 nM vs. ~ 0.07 nM in RM, P and UVP samples). However, methionine sulfone was higher in treated samples following UV treatments. The production of methionine sulfone, as by-product of methionine oxidation, is not reversible unlike the formation of methionine sulfoxide. This effect should be further investigated, as the threshold values for methionine sulfone is not well established in literature for sensory analysis, as it has not been deemed as important a contributor as some of the other protein oxidation products following methionine oxidation. Protein analysis confirmed no significant differences between the α -amino acids and short peptides measured, as well as no differences in the visualisation of the proteins measured under denaturing and non-denaturing conditions. Furthermore quantitative and qualitative measurements on enzyme activity also indicated no differences. The % FFA measured in UVP treatment was higher than the other treatments; however, this can be attributed to mechanical action rather than the direct influence or effect of the UV treatment itself. The analysis of the fatty acid profile indicated difference in C18:1 cis n9 (oleic acid) in the UV sample

and C 18:0 (stearic acid) in the RM sample when compared to the other treatments. The increase in oleic acid in the UV treated sample is different to what was expected, i.e. oxidation that could lead to formation of more saturated fats rather than unsaturated fats. The higher stearic acid levels in the treated samples indicate possible saturation occurring as a result of the treatments received, however, no differences between P, UV and UVP treatments were recorded in this instance.

Sensory analysis did reveal differences between the P and UVP treatments when compared to the UV treatment alone. However, the fact that the UVP rated lower than the UV milk in the 'tallowy' and 'cardboardy' oxidation flavour and aroma descriptors, indicate that it is possible to manipulate these characteristics introduced by UV to some extent by manipulating 'conventional' processing parameters. As a result these flavours could possibly be negated or reduced. The suitability of the milk and final dairy product to consumers, in the authors' opinion, should remain the ultimate test of acceptability, especially in lieu of the advantages alternative processing technologies, such as UV, could provide. The second part of the study is focused on using milk treated with HTST, UV and UV-HTST methods for the manufacturing of Cheddar cheese. It is aimed to provide additional results, especially in regards to the concentration of milk solids and the organoleptic quality of the final product being produced using UV turbulent flow technology as an alternative and/or an adjunct to conventional thermal pasteurisation.

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5.8. Connecting Text

In part 1 of the study, as described in Chapter 5, milk was exposed to a 1 kJ.L⁻¹ of UV-C radiation using a commercial UV system (SurePure SP-40) and the results of the chemical and biochemical analysis were compared to conventional pasteurisation and a combination of UV treatment and pasteurisation. Furthermore the milk processed with each of the treatments was used for the manufacture of Cheddar cheese (Phase 2 of the study) as described in Chapter 6. Concentrated products like Cheddar cheese, provide a model system to study the effects alternative processing technologies, such as UV radiation, as possible ‘defects’ could be amplified with the concentration of nutrient components, in particular fat and protein.

The research results were submitted for publication in the peer-reviewed, scientific journal *Innovative Food Science and Emerging Technologies*:

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Chapter 6

Characterization of Cheddar cheese manufactured from milk treated with heat and ultraviolet (UV) light

6.1. Abstract

Raw milk for commercial production of Cheddar cheese was subjected to three treatments; high temperature short time (HTST) pasteurisation (73.5°C, 15 s) (P), ultraviolet light (1045 J.L⁻¹, 25 s) (UV) and a combination of UV and HTST pasteurisation (1045 J.L⁻¹, 25 s followed by 73°C, 15 s) (UVP). During ripening Cheddar manufactured from UV treated milk showed statistically no significant differences ($p > 0.05$) in the fatty acid profile and lipolysis when compared to the P and UVP variants. A lower level of primary and secondary proteolysis was observed in the UV Cheddar when compared to the P and UVP Cheddar, specifically after 12 months of ripening. UV Cheddar rated superior in organoleptic assessment after 12 months, as the P and UVP variants exhibited bitter notes ($p < 0.05$). In summary, turbulent-flow, thin film UV light systems could provide a viable alternative to traditional thermal processing of cheese milk.

Keywords:

Ultraviolet Light Thin Film Turbulent Flow Treatment; HTST Pasteurisation; Milk; Cheddar cheese; Proteolysis; Lipolysis; Ripening

6.2. Introduction

The production of cheese is an important contributor to economical sustainability of the dairy industry. Approximately one third of the milk produced globally is used for the manufacture of cheese, estimated at approximately 21 million metric tonnes in 2014, with the revenue representing approximately 30% of the total dairy product sales revenue. Cheese consists primarily of fat and coagulated protein and is a highly nutritious food with diverse ranges of flavours and textures (Fox, 1993; De Wit *et al.*,

2005; Andersen *et al.*, 2010). In many instances cheese, as a concentrated dairy product, like milk powder and butter, is also used as a balance for milk preservation as milk is a highly perishable liquid. Cheddar cheese was the obvious choice as a model cheese system to test the influence of UV treatment on the final product characteristics due to its popularity and consumption rate in leading world markets (such as the US and the UK). Furthermore it has been the topic for various peer review articles focussing on the chemical and biochemical changes during ripening.

During manufacture and ripening, cheese production comprises a series of biochemical events, which, if synchronized and balanced, lead to products with highly desirable aromas and flavours, however when unbalanced, results in off-flavours and odours (Fox, 1993; De Wit *et al.*, 2005). Ripening of hard and semi-hard cheese varieties is a long and costly process because of capital immobilization, large refrigerated storage and ripening facilities, weight losses, and spoilage caused by undesirable fermentations due to defective manufacturing conditions or product formulation (Gripón *et al.*, 1991; Fox, 1993; Fox and Wallace, 1997; Beresford & Williams, 2004). Cheese ripening is essentially an enzymatic process that can be divided into three main biochemical events, namely glycolysis, lipolysis, and proteolysis.

Proteolysis is perhaps the most complex and many of the changes that occur during the ripening of cheese can be attributed to the consequences of proteolysis (Law, 2001; Sousa *et al.*, 2001; Fox *et al.*, 2004; Møller *et al.*, 2013). Peptides and amino acids contribute to flavour and textural changes in cheese resulting from the breakdown of the protein network (Andersen *et al.*, 2010). The release of amino and carboxyl groups increase the water binding capacity, therefore also effectively reducing the water activity in the cheese during maturation. Catabolism of free amino acids produces sapid compounds, such as volatile sulphur compounds and fatty acids, and also increases the pH of the cheese through the release of ammonia (Weimer *et al.*, 1999; McSweeney, 2004 *a, b*; Andersen, *et al.*, 2010). The catabolism of especially branched-chain amino acids (BCAA's) and α -keto acid substrates by lactic acid bacteria, such as lactococci and lactobacilli, can lead to the formation of fatty acids (Yvon *et al.*, 1997; Ganesan *et al.*, 2004a; Ganesan *et al.*, 2004b; Ganesan *et al.*, 2004c; Ganesan *et al.*, 2004d). The principal proteolytic agents in cheese are rennet

proteinases (chymosin), indigenous milk proteinases (especially plasmin), and proteinases and peptidases from starter and non-starter bacteria (Andrews, 1983; Fox & Morrissey, 1981; Fox, 1993; Urbach, 1995; Fox, 2003, De Wit *et al.*, 2005). Although of less significance than proteolysis, lipolysis during Cheddar cheese ripening result in the formation of free fatty acids (FFA) (Hickey, *et al.* 2007). FFA's, directly (in particular short and medium chained fatty acids) or indirectly are constituents of Cheddar cheese flavour and can be precursors of flavour compounds such as methylketones, secondary alcohols, esters, aldehydes and lactones (Urbach, 1995; McSweeney *et al.*, 2000; Smit *et al.* 2000; Collins *et al.*, 2003). The various biochemical mechanisms and expected concentration of such secondary metabolites resulting from lipolysis in Cheddar cheese are well described in a review of Collins *et al.* (2003). In total, more than 200 flavour compounds have already been identified in Cheddar cheese, highlighting the significance and importance of balancing the flavour components through correct formulation, manufacturing and storage conditions to produce a safe, palatable, pleasant and acceptable final product to the consumer (Maarse and Visscher, 1996).

During cheese manufacture high temperature short time (HTST) (72°C for 15 s) or low temperature long time (LTLT) (63°C for 30 min) pasteurisation of milk is usually applied to eliminate the risk of growth of vegetative pathogens (Fox *et al.* 2000). Investigation into non-thermal processing technology to conventional heat pasteurisation of milk, such as the use of ultraviolet (UV) irradiation, have gained interest as an alternative or adjunct treatment to thermal pasteurisation, primarily to reduce pathogenic micro-organisms and to extend the shelf-life of the products being produced (Koutchma *et al.* 2004; Koutchma *et al.* 2007; Keyser *et al.* 2007; Matak *et al.* 2005; Reinemann *et al.* 2006). The advantages of non-thermal UV treatment of raw milk during cheese manufacture, would potentially not only be limited to safety and shelf-life benefits due to microbial inactivation and reduction of pathogens, but could possibly be extended further into the production of cheese with an enhanced texture and flavour. Such benefits potentially deliver attractive attributes in the final product, affording manufacturers the opportunity to differentiate their product to compete with cheese manufactured from raw, thermised or pasteurised milk. The hypothesis is that the character of the cheese manufactured from UV treated milk, will more likely resemble the character of cheese produced from raw milk. Cheese makers

often use raw milk for cheese production or add raw milk to pasteurised cheese milk, as it is considered as essential for good texture and flavour. The latter being as a direct result of proteolysis and lipolysis by raw milk microflora (non-starter lactic acid bacteria) and enzymes (Hickey *et al.*, 2007). The UV treatment could potentially limit inactivation of enzymes, such as native lipases and proteases in the milk and reduce the denaturing of the whey proteins, α -lactalbumin (α -LA) and β -lactoglobulin (β -LG), which structure is changed with conventional heat HTST and LTST pasteurisation (Lau *et al.* 1991; Fox *et al.* 2000; Benfeldt and Sorensen, 2001). In addition, UV can also limit defects occurring in the cheese associated with high bacterial counts in raw milk such as flavour defects (fruity, stale, bitter, putrid, rancid), result in increased yield due to the reduction of psychrotrophic bacteria and resulting proteinases from on-farm systems (Richardson, 1983). Thus, the use of UV can result in reducing final product non-conformances.

In part 1 of the study, the effects of heat pasteurisation (P), non-thermal turbulent flow UV treatment (UV) and a combination of the two treatments (UVP) on raw milk in commercial scale Cheddar cheese production were compared (Cilliers *et al.*, 2014). It was concluded in commercial scale that the UV treatment using the SurePure TurbulatorTM alone and in combination with thermal pasteurisation delivered similar antimicrobial efficacy as treatment with conventional HTST alone. Furthermore no significant difference in treatments was observed for macro nutrient composition, except for the beneficial reduction in cholesterol (>18%) in the UV treated milk and a reduction in riboflavin and Vitamin B12 when applying thermal pasteurisation. No significant oxidative deterioration was observed in the lipid and protein fractions when treating the raw milk with UV, although sensory analysis did indicate minor differences in the organoleptic quality, when comparing the UV treated milk to the thermal pasteurised milk (Cilliers *et al.*, 2014).

This study focused on the manufacture of Cheddar cheese from milk treated with UV-C turbulent flow system (SurePure TurbulatorTM, Manufacturer: SurePure, Zug, Switzerland) that has been designed to optimise treatment of turbid fluids. Although the use of UV light for treatment of liquids with low UV transmittance remains challenging, the new generation of UV systems using thin-film turbulent flow regimes

are more efficient once critical design components are optimised (Simons et al, 2013, Koutchma, 2009).

Limited information is available to quantify the effects of UV treatment of milk on the final quality of the cheese in a commercial scale operation. The earlier tests with milk exposed to UV irradiation used much lower UV dosage rates when compared to the SurePure UV system. The applied UV dosage in the studies of Caserio *et al.*, (1975) and Beretta *et al.* (1976) was approximately 5,130 times lower than that of the SurePure UV system tested in this study that makes it difficult to compare the results. Thus, the objective of the second part of the study focused on the chemical, biochemical and organoleptic characterization of Cheddar cheese manufactured from the milk processed by heat pasteurisation, UV light treatment and a combination of UV and heat pasteurisation.

6.3. Materials and Methods

6.3.1. Milk

Raw, full-cream milk (RM) (chilled, co-mingled morning and evening's milk from mixed Friesland and Jersey herds) were used for the manufacture of the Cheddar cheese. Milk was collected from three farmers supplying milk to a commercial cheese plant in Ashton, Western Cape, South Africa. Upon reception, the milk was off-loaded from a tanker and cooled to less than 6°C through a Plate Cooler (Filmatic, Paarl, SA) and stored in a raw milk reception silo before being processed within 24 hours of milking. In each instance 4,000 litres of full-cream raw milk was processed to produce Cheddar cheese. The Cheddar cheese manufactured to conform to the specifications for Cheddar cheese as prescribed in the South African Agricultural Products Standard Act of 1990 (Act 119 of 1990), Regulations Relating to Dairy Products and Imitation Dairy Products (% FIDM 48 – 60 % and minimum % TS of 64%).

6.3.2. Cheese milk treatments

Three different treatments were used to process the RM before the manufacturing of the Cheddar cheese:

Treatment 1 (thermal pasteurisation, P): Was used as the control, RM was thermally pasteurised through a conventional Plate Heat Exchanger (PHE) (Filmatic, Paarl, SA) at 73.5°C for 15 s and then transferred to the cheese vat at 32°C before culture inoculation.

Treatment 2 (UV): The RM was treated with the commercial SP-40 UV system (SurePure AG, Zug, Switzerland) at temperature less than 6°C for 25 s. After treatment milk was heated through a PHE to 32°C and transferred to the cheese vat at 32°C before culture inoculation.

Treatment 3 (UVP): The RM was treated with the SP-40 UV system at less than 6°C followed by immediate thermal pasteurisation at 73.5°C for 15 s and then transferred to the cheese vat at 32°C before culture inoculation.

6.3.3. The novel UV-C system (SurePure Turbulator™)

The commercial SurePure 40 Turbulator™ system (SP-40), as described in the study of Cilliers *et al.* (2014), was used in the study. The commercial SP-40 unit accumulatively delivers a total UV-C dosage of 1045 J.L⁻¹ for 25 s or effective surface UV dose of 430 mJ.cm⁻² determined as a dose in a single turbulator multiplied by a total number of the turbulators (40) connected in series.

6.3.4. Cleaning and Sanitation of the SP-40

The SP-40 system was cleaned before and after every milk treatment using standard ‘*Cleaning in Place*’ (CIP) procedures as described by Cilliers *et al.* (2014).

6.3.5. Cheddar cheese manufacture

Cheddar cheese was manufactured at a commercial cheese plant in Ashton, Western Cape, South Africa according to the standard procedure (Kosikowski, 1982). A schematic flow diagram of the process is given in Figure 6.1.

Following the processing of milk, as described in section 2.2., the cheese milk was inoculated with a standard freeze-dried, direct set mesophilic homofermentative starter culture (RAO 21, Danisco, Denmark) consisting of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Streptococcus thermophilus* at an inoculation rate of 100 DCU's per 1000 L of milk, as per the supplier's specification. At the same time as inoculation, 10 mL of a 30% calcium chloride solution was added to 1000 L of the cheese milk after which a 45 min ripening time was allowed before adding the recombinant chymosin (Maxiren, DSM, The Netherlands) at a dosage rate of 60 mL per 1000 L of cheese milk. Cheeses were manufactured using conventional cheese-making methods; curd was cooked to 38°C, pitched at pH 6.15, milled at pH 5.30, and salted at 2.5% *m/m*.

6.3.6. Sampling methodology

The vacuum packed Cheddar cheeses (10 kg blocks) made were ripened under controlled conditions at approximately 12°C. The cheeses were sampled during ripening, i.e. the day of manufacture, and after 2, 6 and 12 months of ripening. Triplicate samples of the cheese were prepared by aseptically opening the packaging material, representative of each batch, and cutting the sampled portions (100 grams) with a sterile knife if necessary. Samples were frozen at -40°C until chemical analysis, whereas the samples for microbiological analysis were kept on ice at 4°C and analysed within 6 hours of sampling.

6.3.7. Microbial analysis

Cheese samples were plated in triplicate (using serial dilutions 10^{-1} to 10^{-3}) in accordance to methods described in by Wehr and Frank, 2004. Freshly grated cheese

(aseptic technique), was diluted in a ratio of 1:9 *m/m* with sterile 1M phosphate buffer (pH 6.8) which had been pre-heated to 45°C in a water-bath. The mixture was emulsified in a sterile warring blender for 2 min at high speed, taking care that the temperature of the mixture did not exceed 40°C. If required, the pH of the cheese samples was adjusted with 0.1 N NaOH to a pH of 6.80. Standard (Aerobic) plate counts were determined by plating on aerobic plate count Petrifilm™ (3M, Johannesburg, SA) and incubating at 32 ± 1°C for 48 hours. Coliform counts were conducted by plating samples on *E.coli*/Coliform Petrifilm™ (3M, Johannesburg, SA) and incubating at 32°C ± 1°C for 24 hours. Yeast and moulds were determined by plating on yeast and mould plate count Petrifilm™ (3M, Johannesburg, SA) and incubating at 25°± 1°C for 5 days.

6.3.8. Proximate, Chemical and Biochemical Analysis

6.3.8.1. *Proximate Analysis*

Proximate compound analysis on the cheese were performed according to the standards prescribed in “Standard Methods for the examination of Dairy Products”, (Wehr and Frank, 2004). Fat was determined with the Mojonnier ether extraction method (Reference 15.086), Total solids according to the vacuum oven method (Reference 15.111), sodium chloride according to the Mohr method (Reference 15.051), Ash according to the gravimetric method (Reference 15.041) and minerals according to the atomic emission spectroscopic method (Reference 15.101). The % carbohydrates was calculated as the difference between the % TS minus (% fat + % protein + % ash) and would therefore include the % lactic acid.

6.3.8.2. *Extraction of lipids from Cheddar cheese*

Lipids and free fatty acids were extracted from the milk according to the procedure of De Jongh and Badings (1990). A 10 mL milk sample was thoroughly mixed with 10 mL of ethanol (96%) and 1.0 mL of 2.5 M H₂SO₄ for 30 minutes. Subsequent extraction was carried out with a 15 mL ether/heptane (1:1 ratio, *v/v*) in a 100 mL

screw-capped centrifuge tube and then centrifuged at 2500 rpm for three minutes at 20°C. The solvent layer was transferred to a 100 mL conical flask containing 1 g anhydrous Na₂SO₄ to adsorb residual water. The extraction procedure was repeated three times with 15 mL ether/heptane (1:1 ratio, v/v).

6.3.8.3. *Fatty acid analysis*

For the analysis of the fatty acids (FA), the lipid fractions were transesterified to methyl esters in a sodium methylate solution (2 g methanol.l⁻¹). Undecenoic acid (Sigma-Aldrich Corp., St. Louis, MO) was added prior to methylation as an internal standard. Chromatographic analysis was conducted on prepared samples. All samples were analysed in triplicate on a 6890 Hewlett-Packard GC gas chromatograph with a 7683 auto injector, split/splitless capillary injector and flame ionization detector (Agilent Technologies Inc., Sydney, AU). Helium was used as the carrier gas with gas velocity set at 30 cm.s⁻¹, flow rate at 2.2 mL.min⁻¹, injection volume 0.5 µL, and split ratio 100:1. An Agilent CP-FFAP CB, 0.32 mm x 25 m fused silica WCOT FFAP-CB (d_f 0.3 µm) capillary column (Agilent Technologies Inc., Sydney, AU) was used to separate fatty acids and methyl esters. Temperature program for separations began at 40°C, held for 1 min, increased to 100 at 5°C.min⁻¹, held for 3 min, increased to 175°C at 10°C.min⁻¹, held for 45 min, increased to 240°C at 5°C.min⁻¹ and held for 15 min for a total runtime was 87 min. Temperatures for injector and detector were 250 and 300°C, respectively.

6.3.8.4. *Total free fatty acids analysis*

The total free fatty acid (FFA) content was determined by titration according to the AOAC official method of analysis 947.07 by titration of the sample with 1 M NaOH in ethanol (95%) using phenolphthalein as indicator.

6.3.8.5. *Extraction of water-soluble nitrogen and water-insoluble nitrogen in cheese*

The extraction of the water-soluble nitrogen (WSN) and water-insoluble nitrogen (WISN) was done according to the method specified by Kuchroo and Fox (1982). Cheddar cheese (1 part) was mixed with two parts distilled water and homogenized for five minutes with an Ultra-Turrax T25 homogeniser until a smooth paste. The mixture was then heated to 40°C for 1 hour and centrifuged at 3000 x g for 30 minutes (Beckman Centrifuge, Model J2.21). After centrifugation of the homogenate, three fractions were obtained: (1) fat, (2) water soluble extract (supernatant) and (3) the water insoluble extract (precipitate). The WSN extract was filtered through glass wool and the extraction procedure was repeated twice on the precipitate.

6.3.8.6. *Total protein and water soluble nitrogen*

Total protein (TP) analysis of the Cheddar cheese samples was done according to standard IDF procedure (Kjeldahl; IDF, 1964). The water soluble N (WSN) extracts were analysed according to the method of Kuchroo and Fox (1982). The N content of the extracts was determined by the Kjeldahl method and the WSN was expressed as a percentage of total nitrogen.

6.3.8.7. *TNBS method for detection of α -amino acids*

The method of Kuchroo *et al.* (1983) was used for the determination of the α -amino acids. The assay was conducted on the water-soluble fraction. 2,4,6-Trinitrobenzenesulphonic acid (TNBS) is a reagent that reacts specifically with the α -amino group in amino acids for the assessment of proteolysis in cheese. A Cheddar cheese sample (0.5 g) was added to 10 mL borate buffer (0.1 M $\text{Na}_2\text{B}_4\text{O}_7$ in 0.1 M NaOH, pH 9.5). The mixture was warmed to 45°C for 10 min, and then mixed in a glass homogeniser. The homogenate was then centrifuged at 3000 X g for 20 min, after which 3 mL of the aqueous layer was diluted to 250 mL with distilled water. The diluent (0.5 mL) was rapidly mixed with 0.4 mL borate buffer and 1 mL of 0.01% TNBS and incubated at 37°C. The

reaction was terminated after 60 min with 2.0 mL 0.1 M NaH_2PO_4 (containing 1.5 mM Na_2SO_3) and the absorbance read at 420 nm recorded.

6.3.8.8. *SDS- and Urea Polyacrylamide gel electrophoresis (PAGE)*

SDS-PAGE gels were performed using the method of Andrews, (1983). Urea-PAGE gels were performed according to the method of Shalabi and Fox, (1987).

Sample preparation for the RP-HPLC was done according to the method of Law *et al.* (1992). Lyophilized samples of the WSN fraction were dissolved in deionized water (Milli-Q water) (5 mg.mL^{-1}), and filtered through a $0.45 \mu\text{m}$ pore size pre-filter. HPLC was carried out on an HP/Agilent system with a PDA detector, operated at 220 nm. Samples were chromatographed on a $5 \mu\text{m}$ particle size Jupiter RP C_{18} column (250 mm x 4.6 mm equipped with a Jour-Guard RP/C18 - $5\mu\text{m}$ guard column at ambient temperature. A gradient elution was performed with the following eluents: Eluent A (0.15% TFA, (ion-pairing agent) in DDW) and eluent B (0.1% TFA (Sigma, Johannesburg, South Africa) in 80:20 (v/v) acetonitrile (Sigma, Johannesburg, South Africa) in DDW). A linear gradient was run from 100% A to 30% A over 70 min at a flow rate of 1 mL.min^{-1} . All solvents were degassed under vacuum before use.

6.3.9. Organoleptic Analysis

During ripening at the specified intervals (2, 6 and 12 months) the Cheddar cheese were blind graded in accordance with the guidelines of the American Dairy Science Association (ADSA) as described by Bodyfelt *et al.* (1988). Eight trained panellists (University of Stellenbosch, Stellenbosch and DuPont Innovation, Cape Town, South Africa) were used to evaluate the Cheddar cheese. Grading was done according to the criteria and parameters used during the Annual South African National Cheese Championships (Agri-Expo, Durbanville, South Africa). Cheese is graded and points are awarded for appearance (maximum 2 points), body and texture (maximum 7 points) and flavour (maximum 11 points). The points awarded per individual Cheddar cheese are tallied to give a final rating (maximum 20 points).

6.3.10. Statistical Analysis

One-way analyses of variance (ANOVA) was performed using data from triplicate samples of the three Cheddar cheeses manufactured using different treatments during the different ripening periods of 0, 6 and 12 month's respectively. In the instance where the use of ANOVA and t-test analysis was deemed not to be suitable due not adhering to certain criteria, a robust statistical method Median Polishing (MP), developed by Tukey, was used (Hoaglin, Mosteller & Tukey, 1983). The criteria that were not satisfied in the instances ANOVA were not used, include: (1) independence of the samples, (2) equal variances and (3) normality. MP is fitting a model and can be explained with the following equation:

Equation 7
$$y_{ij} = m + r_i + c_j + e_{ij}$$

Where y_{ij} is the value in row and column, m is a value common to all cells, r_i is the row effect, c_j is the column effect and e_{ij} the error term in each of the cells. The MP technique uses the median rather than the mean with the result that the method is robust to outliers, the influence is less than for mean tests. The ProphetStat statistical program was used for the analysis.

ANOVA was performed on the data using SAS version 8.12. Student's t-least significant difference (LSD) was calculated at the 5% significance level to compare treatment means.

6.4. Results and Discussion

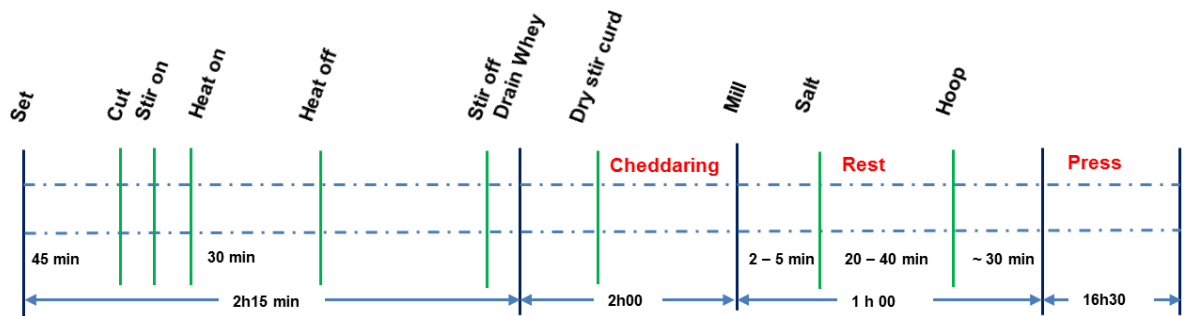
6.4.1. Effect of thermal and UV treatment on Cheddar cheese production parameters

The basic process of Cheddar cheeses manufactured is summarized in Figure 6.1. During the commercial production of Cheddar cheese from UV treated milk, a reduction in renneting time (RT) of approximately 27% was observed. The RT was defined as the time in minutes from addition of the rennet to when the curd was

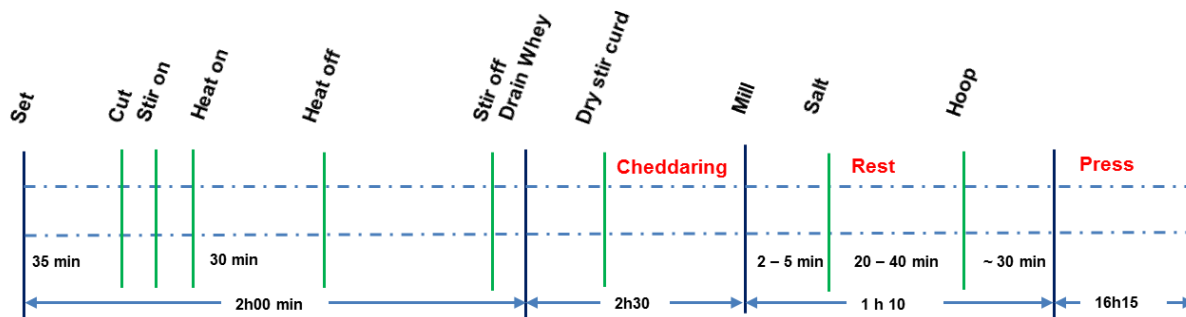
deemed firm enough to cut. The reduction in RT could be due to partial insolubilisation of the colloidal calcium phosphate during heat treatment, thus reducing the coagulation properties of the milk (Fox and McSweeney, 1998). The results of the commercial trial correlated well with our previous experimental findings that indicated a reduction of between 21 – 23% in the rennet coagulation time (RCT) of UV treated milk compared to pasteurised milk (results not shown). The reduction in RCT was determined with stress rheometry, and is an accepted index of the coagulation properties of milk and there would be a direct correlation between RCT and RT (Fox and McSweeney, 1998). The RT could be adjusted and optimised by adjusting the rennet concentration added to the cheese milk and/or adjusting the CaCl_2 concentration added to the cheese milk and could be the subject for future studies. Both these practises could provide advantages for the cheese maker from an economic impact point in the instance where less rennet is added and from a reduction of possible organoleptic defects in the final product (such as bitterness), by reducing the amount of CaCl_2 added. However, caution should be exercised in adjusting the rennet concentration as the rennet will also contribute to proteolysis during ripening. It was found that during the stirring of the curd, the UV treated milk produced a softer, higher moisture curd. As a result the dry stirring of the curd was extended to achieve favourable curd rheology before milling and pressing. Some free oil was noticed on the milk surface during the manufacture of the UVP variant when the culture was added. The cause of the free oil could possibly be due to excessive shear causing damage to the milk fat globule membranes during the pumping of milk through the SP-40 UV system and PHE. This defect could negatively impact on the yield of the cheese, and increase the probability of development of undesirable off-flavour during ripening due to the damage of the milk fat globule membrane. This defect could possibly be avoided by the adjusting the flow rate of the milk.

The theoretical cheese yield calculated was 10.7% using the formula of Van Slyke and Price (1952). The actual yields were higher than calculated yield for all three treatments, with the UV treatment exhibiting the highest yield percentage of 13.2%. When heat pasteurisation was applied alone or combination with UV, both had similar cheese yield, measuring at 12.5% and 12.5% for the P and UVP treatments respectively.

(a) P



(b) UV



(c) UVP

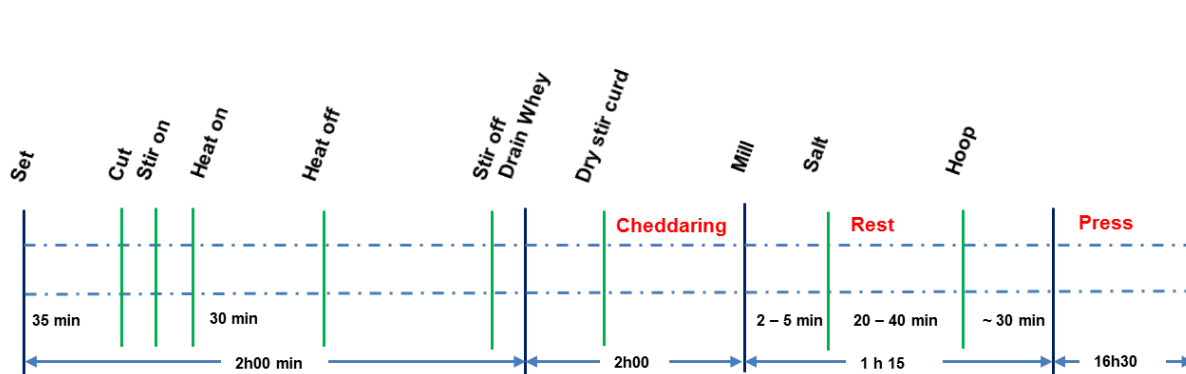


Figure 6.1: Schematic flow diagram of the Cheddar cheese manufacturing process for (a) P, (b) UV and (c) UVP.

6.4.2. Effect of thermal and UV treatment on Cheddar cheese micro flora

The microbial counts of the Cheddar cheese manufactured with the milk treated by P, UV and UVP treatments, conformed to the following microbial specifications on the day of production (day 0): coliform bacteria, $< 500 \text{ cfu.g}^{-1}$; *E.coli*, 0 cfu.g^{-1} and yeast and moulds, $< 200 \text{ cfu.g}^{-1}$.

6.4.3. Effect of thermal and UV treatment on Cheddar cheese composition

6.4.3.1. *Macronutrient components, and physico-chemical parameters*

The results of the macronutrient composition of the Cheddar cheeses produced using milk treated by the P, UV and UVP immediately after treatments (day 0) and during ripening are summarised in Table 6.1. The Cheddar cheeses analysed at day 0 and after 6 and 12 months of ripening showed little variation in terms of the composition at the specified time interval. At specific intervals during ripening (i.e. day of production, 6 and 12 months), it was noteworthy that statistical analysis (both on MP and regression analysis) did not indicate any significant differences ($p > 0.05$) between macro nutrient component values (% fat, % protein and % total solids) of the Cheddar cheese produced with the P, UV and UVP treated milk. Therefore it can be concluded that as far as the macro-components are concerned, no differences were observed between the three treatments at any specific time interval.

However, the MP model did indicate deviations on some of macronutrients in cheese following the three treatments during the complete 12 month ripening period when comparing analysis between different time intervals, as would be expected. Most noticeable differences highlighted, according to the MP, were the % fat, % monounsaturated fat (MUF) and % moisture (samples UV_0 and UV_6 , $p < 0.05$). The moisture loss of 10.2% was the largest for the UVP treatment after 12 months, followed by P (7.3%) and UV treatment (6.3%). Although the moisture loss seems high, the results correlate well with general observations during the ripening of cheese, especially semi-hard and hard variants such as Cheddar.

Table 6.1: Chemical Composition of Cheddar cheese: Day of Production, 6 months and 12 month

Nutrient	P ₀		UV ₀		UVP ₀		P ₆		UV ₆		UVP ₆		P ₁₂		UV ₁₂		UVP ₁₂	
(g.100g ⁻¹)	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	UVP ₁₂	±SD
Fat (Base Hydrolysis)	32.37	0.43	33.69	0.44	32.26	0.38	34.79	0.42	36.21	0.37	34.68	0.33	34.09	0.41	35.82	0.30	34.40	0.27
<i>Saturated Fat</i>	24.86	0.23	25.67	0.18	24.62	0.29	26.62	0.38	27.38	0.31	25.28	0.29	25.30	0.28	26.58	0.23	24.70	0.30
<i>Monounsaturated Fat</i>	5.86	0.22	6.27	0.28	5.90	0.22	6.33	0.28	6.74	0.17	7.42	0.13	6.65	0.23	6.99	0.17	7.64	0.19
<i>Polyunsaturated Fat</i>	0.71	0.03	0.78	0.03	0.71	0.04	0.77	0.03	0.83	0.04	0.76	0.05	0.78	0.02	0.82	0.03	0.79	0.04
<i>Trans Fatty Acids</i>	0.94	0.03	0.91	0.03	1.03	0.04	1.08	0.03	1.12	0.03	1.18	0.03	1.06	0.03	1.11	0.02	1.07	0.02
<i>Miscellaneous</i>	0.00	0.00	0.07	0.00	0.00	0.01	0.04	0.01	0.15	0.01	0.07	0.03	0.31	0.01	0.32	0.02	0.21	0.01
Protein (6.38)	22.00	0.03	21.10	0.02	21.80	0.02	21.40	0.02	20.70	0.03	22.00	0.03	21.40	0.04	21.80	0.04	22.40	0.02
Total Carbohydrate	2.69	0.06	3.91	0.03	3.06	0.02	3.91	0.04	4.02	0.06	3.59	0.04	4.81	0.05	3.61	0.04	4.37	0.02
Ash	3.90	0.07	4.00	0.04	4.00	0.03	3.90	0.04	3.90	0.05	3.90	0.04	3.50	0.045	3.80	0.04	3.90	0.04
Moisture (Vacuum)	39.05	0.05	37.30	0.05	38.88	0.04	36.00	0.05	35.17	0.05	35.83	0.03	36.20	0.04	34.97	0.04	34.93	0.03
Total Solids (DM)	60.95	0.06	62.70	0.05	61.12	0.04	64.00	0.04	64.84	0.05	64.17	0.04	63.80	0.03	65.03	0.03	65.07	0.02
Fat in Dry Matter	53.10	0.04	53.73	0.40	52.79	0.35	54.36	0.03	55.85	0.28	54.05	0.50	53.43	0.50	55.08	0.39	52.87	0.24
Salt	1.33	0.04	1.40	0.04	1.35	0.04	1.40	0.04	1.50	0.04	1.60	0.02	1.60	0.02	1.60	0.02	1.50	0.03
Salt in Moisture	3.41	0.06	3.75	0.07	3.47	0.06	3.89	0.04	4.27	0.05	4.47	0.05	4.42	0.05	4.58	0.04	4.29	0.03

P – Treatment 1: Pasteurised Milk; P₀ at day of production, P₆ after 6 months ripening, P₁₂ after 12 months of ripening

UV – Treatment 2: UV treated Milk; UV₀ at day of production, UV₆ after 6 months ripening, UV₁₂ after 12 months of ripening

UVP – Treatment 3: UV and Pasteurised Milk; UVP₀ at day of production, UVP₆ after 6 months ripening, UV₁₂ after 12 months of ripening

6.4.3.2. *Fat, Fatty Acids and Free Fatty Acids*

The role of lipolysis and flavour development during the ripening of Cheddar cheese is of secondary importance when compared to proteolysis (Woo & Lindsay, 1982; Woo & Lindsay, 1984; Brennand *et al.* 1989). However, the role of lipolysis of milk-fat within flavour development should not be underestimated as it has been reported that low-fat Cheddar cheese with lower concentration of FFA's lacks typical Cheddar cheese flavour development during maturation (Collins *et al.*, 2003).

According to MP statistically significant differences were recorded when comparing the % fat of the different Cheddar cheeses produced, as indicated in Table 6.1. Although the fat content of the cheese samples of P₀ and UVP₀ treatment were comparable, the MP indicated significant differences when compared to UV₀, therefore it seems that the UV treatment alone did yield cheese with a slightly higher fat content. This finding was attributed to less fat loss in the whey during UV₀ treatment of milk and a subsequent better fat recovery in the UV₀ cheese samples (% fat in whey: P₀ whey 0.48%, UV₀ whey 0.27%, UVP₀ whey 0.55%). This finding is also consistent with the results of fat content in Cheddar cheese after 8-weeks, 6 months and 12 months indicating the higher % fat in the UV treatments compared to P and UVP treatments.

A statistically significant decrease of FA content over time ($p < 0.05$) was observed on all variants, most noticeable on the UVP₆ and UVP₁₂ as per the MP analysis. These findings are consistent with the observations by El-Tanboly *et al.* (2000), whom reported the disappearance of some FA during normal ripening and the formation of secondary metabolites, such as methyl ketones, lactones, esters and secondary alcohols, during cheese ripening (Collins *et al.*, 2003; Hickey *et al.*, 2007). Further investigation is needed to quantify the levels of secondary metabolites produced from FAs during ripening to ascertain whether there are any differences in the concentration of individual compounds as a result of the respective treatments. In contrast, no statistically significant differences between the FA measured at any given time interval between the three different treatments Cheddar cheese produced, were recorded ($p > 0.05$) (Table 6.2).

Table 6.2: Fatty acid analysis % w/w of Cheddar cheese: Day of Production (Day 0), 6 months (180 days) and 12 months (360 days).

Fatty Acids	P ₀		UV ₀		UVP ₀		P ₆		UV ₆		UVP ₆		P ₁₂		UV ₁₂		UVP ₁₂	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
C4:0 (Butyric)	5.80	0.33	5.50	0.20	5.90	0.29	5.80	0.41	5.60	0.23	3.20	0.23	4.60	0.28	4.60	0.17	3.40	0.22
C6:0 (Caproic)	3.90	0.17	3.70	0.20	4.00	0.23	3.90	0.29	3.70	0.33	2.50	0.09	3.10	0.13	3.10	0.20	2.40	0.13
C8:0 (Caprylic)	2.30	0.17	2.20	0.11	2.40	0.28	2.30	0.09	2.20	0.09	1.60	0.10	1.90	0.15	1.90	0.23	1.50	0.10
C10:0 (Capric)	4.70	0.22	4.70	0.32	4.80	0.28	4.70	0.21	4.70	0.18	3.50	0.15	4.10	0.19	4.10	0.18	3.40	0.23
C10:1	0.50	0.03	0.50	0.03	0.50	0.03	0.50	0.03	0.50	0.05	0.40	0.03	0.50	0.04	0.50	0.04	0.40	0.04
C12:0 (Lauric)	5.20	0.32	5.20	0.28	5.10	0.26	5.10	0.33	5.20	0.26	4.20	0.30	4.70	0.32	4.70	0.17	3.90	0.18
C12:1	0.30	0.02	0.30	0.02	0.30	0.03	0.30	0.02	0.30	0.04	0.20	0.02	0.30	0.05	0.30	0.03	0.30	0.04
C13:0 (Tridecanoic)	0.10	0.01	0.10	0.01	0.10	0.01	0.10	0.01	0.10	0.03	0.10	0.05	0.10	0.07	0.10	0.03	0.10	0.02
C14:0 (Myristic)	14.20	0.69	14.00	0.56	13.80	0.69	13.90	0.68	14.00	0.68	12.60	0.66	13.50	0.81	13.50	0.77	12.10	0.55
C14:1 (Myristoleic)	0.70	0.10	0.80	0.04	0.70	0.05	0.70	0.04	0.70	0.05	0.60	0.04	0.80	0.03	0.80	0.05	1.00	0.05
C15:0 (Pentadecanoic)	1.40	0.09	1.30	0.07	1.30	0.03	1.30	0.07	1.30	0.05	1.30	0.05	1.40	0.04	1.40	0.06	1.30	0.05
C15:1 (cis-10-Pentadecanoic)	0.20	0.05	0.20	0.01	0.20	0.01	0.20	0.01	0.20	0.03	0.20	0.04	0.30	0.09	0.30	0.02	0.30	0.04
C16:0 (Palmitic)	29.50	1.56	29.50	1.64	29.00	1.53	29.30	1.47	29.10	1.74	30.20	1.64	29.80	1.63	29.80	1.54	29.50	1.36
C16:1 (Palmitoleic)	1.50	0.05	1.50	0.08	1.40	0.07	1.50	0.08	1.50	0.07	1.60	0.09	1.60	0.12	1.60	0.08	1.60	0.08
C17:0 (Heptadecanoic iso)	0.50	0.03	0.50	0.03	0.50	0.03	0.50	0.03	0.50	0.10	0.60	0.04	0.60	0.04	0.60	0.03	0.70	0.05
C17:0 (Heptadecanoic anteiso)	0.50	0.03	0.50	0.03	0.50	0.03	0.50	0.02	0.50	0.09	0.50	0.02	0.50	0.04	0.50	0.03	0.60	0.09
C17:0 (Heptadecanoic)	0.10	0.01	0.10	0.03	0.10	0.01	0.10	0.02	0.10	0.02	0.10	0.01	0.20	0.02	0.20	0.01	0.20	0.03
C17:1 (cis-10-Heptadecanoic)	0.10	0.01	0.10	0.05	0.10	0.01	0.10	0.02	0.10	0.01	0.20	0.01	0.20	0.02	0.20	0.01	0.20	0.03
C18:0 (Stearic)	8.30	0.39	8.30	0.33	8.50	0.22	8.30	0.40	8.20	0.44	12.00	0.58	9.20	0.49	9.20	0.49	12.00	0.56
C18:1 cis n9c (Oleic acid)	14.00	0.32	14.40	0.87	14.30	0.89	14.20	0.81	14.40	0.75	17.40	0.93	15.40	0.69	15.40	0.83	17.70	0.88
C18:1 trans n9t (Elaidic)	0.70	0.04	0.80	0.04	0.70	0.04	0.70	0.07	0.90	0.06	0.80	0.02	0.50	0.04	0.50	0.05	0.80	0.04
C18:2 n6c (Linoleic acid)	1.70	0.07	1.90	0.10	1.70	0.14	1.70	0.06	1.90	0.14	1.80	0.07	1.80	0.07	1.80	0.05	1.90	0.10
C18:2 Conjugated CLA	0.10	0.01	0.10	0.01	0.10	0.01	0.10	0.02	0.10	0.05	0.10	0.04	0.10	0.02	0.10	0.05	0.10	0.01
C18:3 3n3 (alpha-Linolenic acid)	0.50	0.03	0.50	0.03	0.50	0.03	0.50	0.02	0.50	0.10	0.40	0.03	0.50	0.11	0.50	0.04	0.40	0.03
C20:0 (Arachidic)	0.40	0.02	0.40	0.02	0.40	0.02	0.40	0.02	0.40	0.02	0.50	0.02	0.50	0.04	0.50	0.03	0.60	0.03
C:22:0 (Behenic)	0.10	0.01	0.10	0.01	0.10	0.03	0.10	0.05	0.10	0.12	0.10	0.01	0.10	0.06	0.10	0.01	0.10	0.01
<i>Total Trans Fatty Acids</i>	<i>2.90</i>	<i>0.14</i>	<i>2.70</i>	<i>0.15</i>	<i>3.20</i>	<i>0.11</i>	<i>3.10</i>	<i>0.08</i>	<i>3.10</i>	<i>0.20</i>	<i>3.40</i>	<i>0.14</i>	<i>3.10</i>	<i>0.13</i>	<i>3.10</i>	<i>0.20</i>	<i>3.10</i>	<i>0.11</i>
<i>Total Saturated Fat</i>	<i>76.80</i>	<i>3.39</i>	<i>76.20</i>	<i>3.43</i>	<i>76.30</i>	<i>3.45</i>	<i>76.50</i>	<i>3.92</i>	<i>75.60</i>	<i>3.73</i>	<i>72.90</i>	<i>3.72</i>	<i>74.20</i>	<i>3.43</i>	<i>74.20</i>	<i>3.65</i>	<i>71.80</i>	<i>3.60</i>
<i>Total Mono-Unsaturated Fat</i>	<i>18.10</i>	<i>1.00</i>	<i>18.60</i>	<i>0.79</i>	<i>18.30</i>	<i>0.78</i>	<i>18.20</i>	<i>0.90</i>	<i>18.60</i>	<i>0.77</i>	<i>21.40</i>	<i>1.01</i>	<i>19.50</i>	<i>0.78</i>	<i>19.50</i>	<i>1.03</i>	<i>22.20</i>	<i>1.12</i>
<i>Total Poly-Unsaturated Fat</i>	<i>2.20</i>	<i>0.13</i>	<i>2.30</i>	<i>0.11</i>	<i>2.20</i>	<i>0.09</i>	<i>2.20</i>	<i>0.09</i>	<i>2.30</i>	<i>0.14</i>	<i>2.20</i>	<i>0.05</i>	<i>2.30</i>	<i>0.12</i>	<i>2.30</i>	<i>0.06</i>	<i>2.30</i>	<i>0.10</i>
<i>Miscellaneous</i>	<i>0.20</i>	<i>0.01</i>	<i>0.20</i>	<i>0.01</i>	<i>0.20</i>	<i>0.01</i>	<i>0.20</i>	<i>0.03</i>	<i>0.40</i>	<i>0.02</i>	<i>0.20</i>	<i>0.03</i>	<i>0.90</i>	<i>0.07</i>	<i>0.90</i>	<i>0.08</i>	<i>0.60</i>	<i>0.03</i>

Free fatty acids (FFAs) vary between cheese varieties, however, it should not exceed 2% of the triacylglycerides in cheeses such as Gouda and Cheddar (Gripson, 1993). Too high concentration of FFA can lead to undesirable defects in Cheddar cheese such as rancidity or ‘soapiness’. On average a greater than 50% reduction in FFA’s after 6 and 12 months’ ripening on all the Cheddar cheeses produced, were observed when compared to FFA’s on the day of production. The reduction in FFA’s, as is the case with the FA’s, could be as a result of the formation of secondary metabolites produced through catabolism of the fatty acids (Molimard and Spinnler, 1996). No significant differences in FFA concentration between the different treatments were observed during maturation at a specific time interval ($p > 0.05$). After 12 months the final concentration of FFAs was $0.14 \pm 0.01\%$, $0.10 \pm 0.04\%$ and $0.13 \pm 0.04\%$ respectively for the P₁₂, UV₁₂ and UVP₁₂ variants, as indicated in Figure 6.2. These values are consistent with reports that the average percentage of free fatty acids in matured Cheddar cheese is approximately between 0.10% - 0.25% (Woo *et al.* 1984; Ikens *et al.*, 1988; McSweeney & Sousa, 2000). Various factors typically influence the formation of FFA in Cheddar cheese, i.e. type of milk treatment, starter culture and/or adjuncts used, the indigenous lipase activity of the milk, lipase and esterases arising from micro-organisms in the milk and lipase in the rennet (McSweeney & Sousa, 2000; Fox *et al.*, 2004).

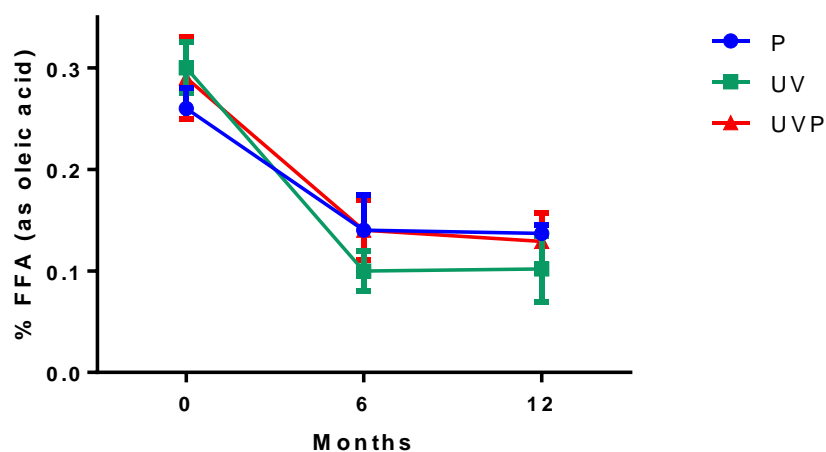


Figure 6.2: Free fatty acids expressed as % w/w per kg of cheese during 0, 6 and 12 month ripening of Cheddar cheese produced by HTST, UV turbulent flow treatment and UV treatment followed by HTST treatment. Error bars indicates standard deviation.

6.4.3.3. *Proteolysis*

Proteolysis is the major biochemical event in ripening of most cheese varieties as it affects both the body, texture and the flavour of the final product (Kuchroo *et al.* 1982; Kuchroo *et al.* 1983, Folkertsma and Fox 1992). The level of proteolysis is generally considered as the main criteria determining the degree of ripening, serves as an indicator for assessing acceleration of cheese ripening and as a tool for starter culture selection (Fox, 1989). Primary proteolysis, as detected by PAGE, is the hydrolysis of proteins to smaller water-soluble and larger water insoluble peptides. Secondary proteolysis is the catabolism of the peptides to smaller peptides, amino acids and proteins that will be present in the WSN, mainly as a result of the action of starter and non-starter proteinases and peptidases (Rank *et al.* 1985; Shakeel-Ur-Rehman *et al.*, 2003).

The results of the Kjeldahl analysis on the Cheddar cheese did not indicate any significant differences ($p > 0.05$) in total protein nitrogen (TPN) measured during ripening after all milk treatments as indicated in Table 6.1. In contrast, as would be expected and indicated in Figure 6.3, the water soluble nitrogen (WSN) as a percentage of total nitrogen (TN) does indicate a significant increase ($p < 0.05$) on all variants during ripening, especially until 6-months from production, which indicates that proteolysis has occurred in all samples of Cheddar cheeses produced. These results are in agreement with reports from Farkye and Fox (1990) and Law *et al.* (1992). However, there were no significant differences ($p > 0.05$) between the three milk treatments at any specific time interval, i.e. day of production, 6 months or 12 months. The increase in WSN (smaller peptides, amino acids) in the Cheddar cheese can be attributed to a combination of factors such as proteolysis by plasmin, residual rennet activity and normal proteases produced by the homo-fermentative starter culture used. This is necessary for normal flavour development in Cheddar, especially matured cheese, where controlled proteolysis gives a certain character to the cheese. As indicated in Figure 6.3 an increase in the WSN: TN was observed from approximately 4.5% to $> 30\%$ on all three cheese variants produced after 12 months of ripening. The WSN: TN for the UV₁₂ sample ($32\% \pm 5\%$), was slightly lower than that of the P₁₂ ($35 \pm 5\%$) and UVP₁₂ ($35 \pm 3\%$) treatments of milk, but this difference was not statistically significant ($p > 0.05$). It is important to take in

consideration that the WSN: TN only gives information about the extent of proteolysis, and no detail of the composition of the WSN fraction. It would therefore be possible that proteolysis in the three treatments resulted in different breakdown products, although the total amounts of these products were approximately the same, and therefore would need to be further investigated.

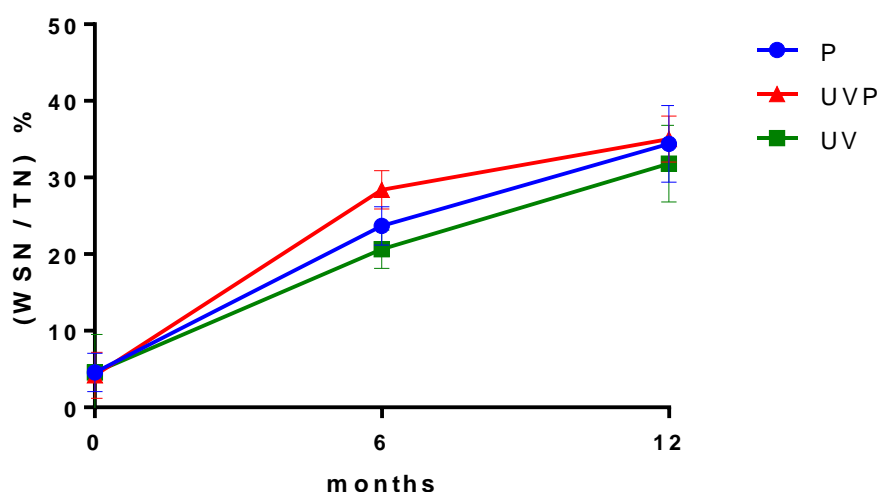


Figure 6.3: Water soluble nitrogen (WSN): as a percentage of Total Nitrogen % w/w during the ripening of Cheddar cheese following HTST, UV turbulent flow treatment and UV treatment followed by HTST treatment. Error bars indicates standard deviation.

Figure 6.4 shows the results of the cheese analysis using the TNBS method in which the extent of primary proteolysis was quantified on the WSN fraction. TNBS identifies both the α - and ϵ -amino groups of proteins and peptides to produce a coloured complex absorbing maximally at 420 nm (Kuchroo *et al.* 1983). The extent of proteolysis, as measured by the TNBS method, is dependent on various factors, such as the initial quality of the milk, type of culture or adjunct cultures used, rennet, type and age of cheese. The TNBS values were expressed as mg Leu.g^{-1} cheese. The normal range for Cheddar cheese reported in the literature varies between 1.8 mg.g^{-1} (0-1 month of ripening) to 25 mg.g^{-1} of Cheddar cheese (after 12 months of ripening) (Aston *et al.* 1983). The values recorded during ripening of the UV-treated milk were lower than the P and UVP treatments after 6 and 12 months, although the initial

values for all three variants were identical at the day of production. The analytical values of the UV₆ and UV₁₂ cheese samples were approximately 25% lower than the P₆, P₁₂ and UVP₆ and UVP₁₂ samples throughout ripening, increasing from 2.0 mg.g⁻¹ (day 0) to 3.1 mg.g⁻¹ (after 12 months). The profiles and rate of amino acids formed in the P and UVP variants were very similar, with the concentration on both these variants being exactly the same after 12 months ripening. It was concluded that the free amino acid concentration measured for the UV treatment sample was significantly lower than that of P and UVP treatment samples ($p < 0.05$). Possible explanations for this observation include an increased whey protein denaturation with heat treatment which could serve as a more suitable substrate for proteolysis by endopeptidases and proteases, when compared to non-heat treated milk (Snoeren en Both, 1981; Grufferty and Fox, 1988). Furthermore, the rate of catabolism of amino acids to produce flavour compounds (secondary metabolites) through various biochemical pathways and enzymatic action (i.e. such as deaminases, decarboxylases, transaminases, aminotransferases and lyases) could also be a potential point of difference between the treatments (Yvon and Rijnen, 2001). The composition of the WSN fraction in such an instance would greatly impact on the rate of conversion of amino acids to secondary metabolites and volatile flavour compounds (Urbach, 1995; Engels *et al.* 1997). These points would, however, need further investigation and elucidation.

SDS-PAGE is a technique commonly used to assess the extent of primary proteolysis based on the separation of the proteins on their size and charge. According to Grappin *et al.* (1985) chymosin is the principal proteolytic agent responsible for proteolysis detected by gel electrophoresis, especially during the early stages of ripening. The SDS-PAGE of the WISN will deliver similar results to that of the complete fractionated cheese; hence emphasis on differences obtained in the WSN fractions will be of greater importance in an assessment of proteolysis (Shalabi and Fox, 1987). SDS-PAGE will not be as effective for the separation of the 4 types of casein, as they have very similar molecular weights. Furthermore, due to the high surface hydrophobicity of β -casein, it also binds a disproportionately high quantity of SDS, and as result has a higher electrophoretic mobility than α s₁-casein, although it is a larger molecule.

UREA-PAGE analysis will provide better resolution of proteins when compared to SDS-PAGE, as the molecular weight of the proteins is similar (between 20 – 25 kDa). However, SDS-PAGE still provides valuable information as far as the ripening profile of cheese is concerned. The UREA-PAGE gels of the WSN and WISN demonstrated a decrease in α ₁-casein, β -casein (Figure 6.5*a* and Figure 6.5*b*). SDS-PAGE of the WSN fraction also indicated a decrease in β -LG over ripening in all treatments with no significant differences observed in electrophoretic patterns in these fractions between the P, UV and UVP treatments, mainly attributed to plasmin activity. Furthermore an increase in the proteose peptone and smaller proteins were observed for all treatments after 6 and 12 months in the WSN fraction, indicative of proteolysis and the formation of smaller peptides (γ -casein), mainly due to the hydrolysis of casein by chymosin and plasmin. It should also be considered that this fraction will also contain minor proteins indigenous to milk, e.g osteopontinin and trace amounts of lactosylated α -LA and β -LG. The formation of smaller peptides and molecules is of significance in the WSN fraction, as the WSN retains flavour components as supposed to the WISN, which is flavourless.

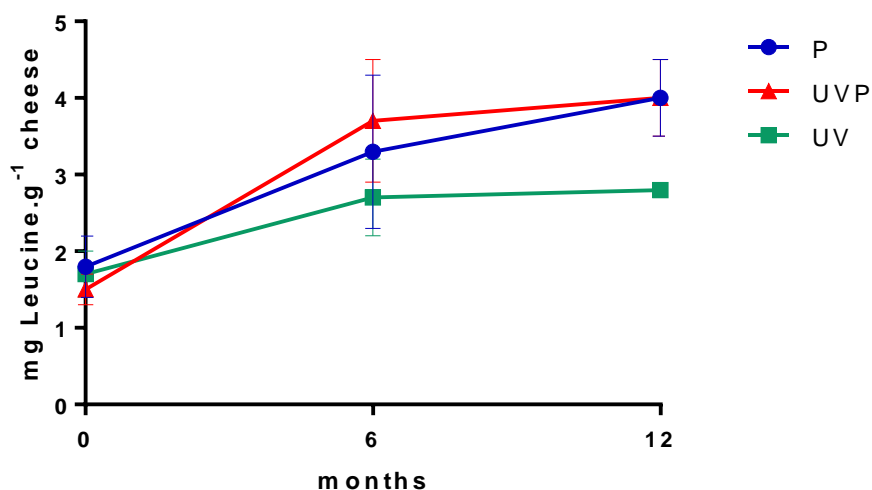


Figure 6.4: Total free amino acids (mg Leu.g⁻¹ of cheese) during different stages of ripening according to the TNBS method of the WSN fraction of Cheddar cheese produced by HTST, UV turbulent flow treatment and UV treatment followed by HTST treatment. Error bars indicates standard deviation.

Figure 6.5a

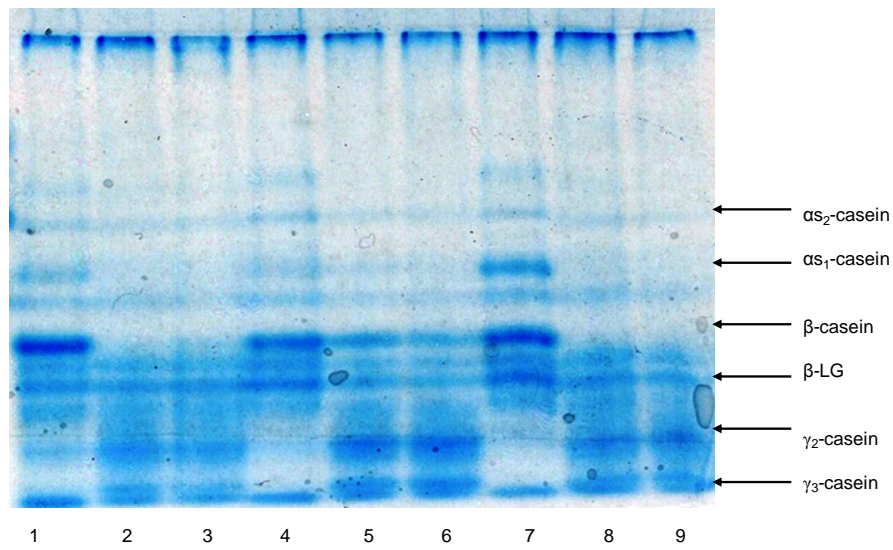


Figure 6.5b

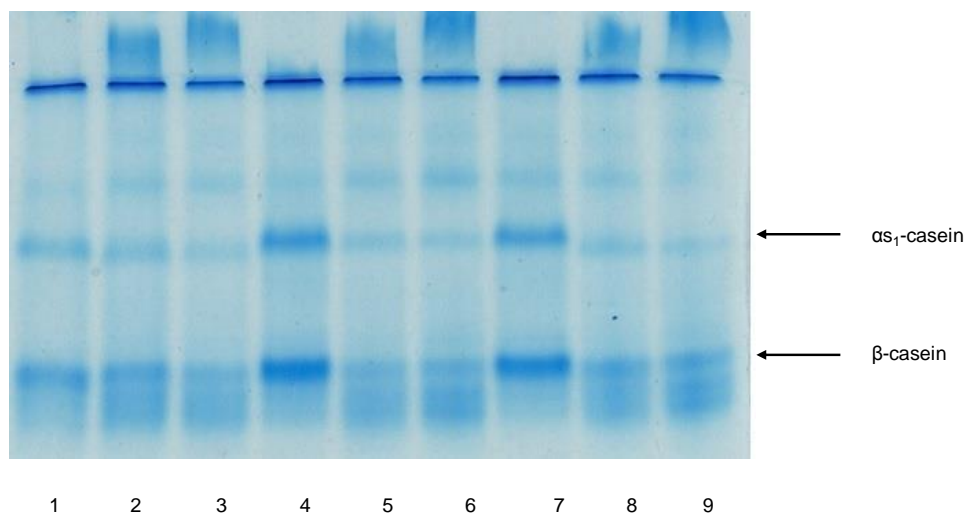


Figure 6.5a & b: SDS-PAGE of WSN fraction (a) and WISN (b) in Cheddar cheese during different stages of ripening. Lane 1- P_0 ; Lane 2; P_6 ; Lane 3 – P_{12} ; Lane 4; UVP_0 ; Lane 5 – UVP_6 ; Lane 6 – UVP_{12} ; Lane 7 – UV_0 ; Lane 8 – UV_6 ; Lane 9 – UV_{12} .

UREA PAGE analysis of the WSN and WISN indicated hydrolysis of β -casein increasing during cheese ripening on all treatments (Figure 6.6a and Figure 6.6b). However, no significant differences in electrophoretic patterns were observed between the different treatments during ripening. The α ₁-casein was hydrolysed during ripening on all variants, mainly due to proteolysis of the α ₁-casein by chymosin. There was a definite reduction in β -casein which was almost completely hydrolysed after 12 months of ripening on all three treatments with no visible differences between different samples.

Figure 6.6 a

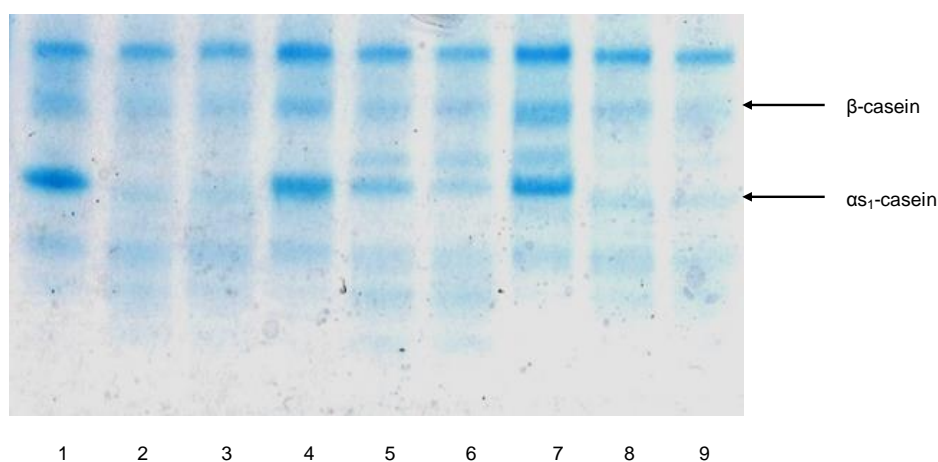


Figure 6.6b

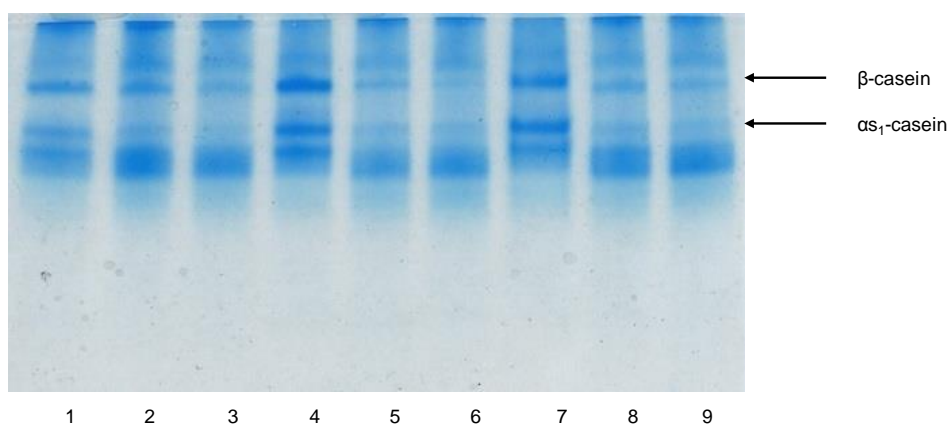
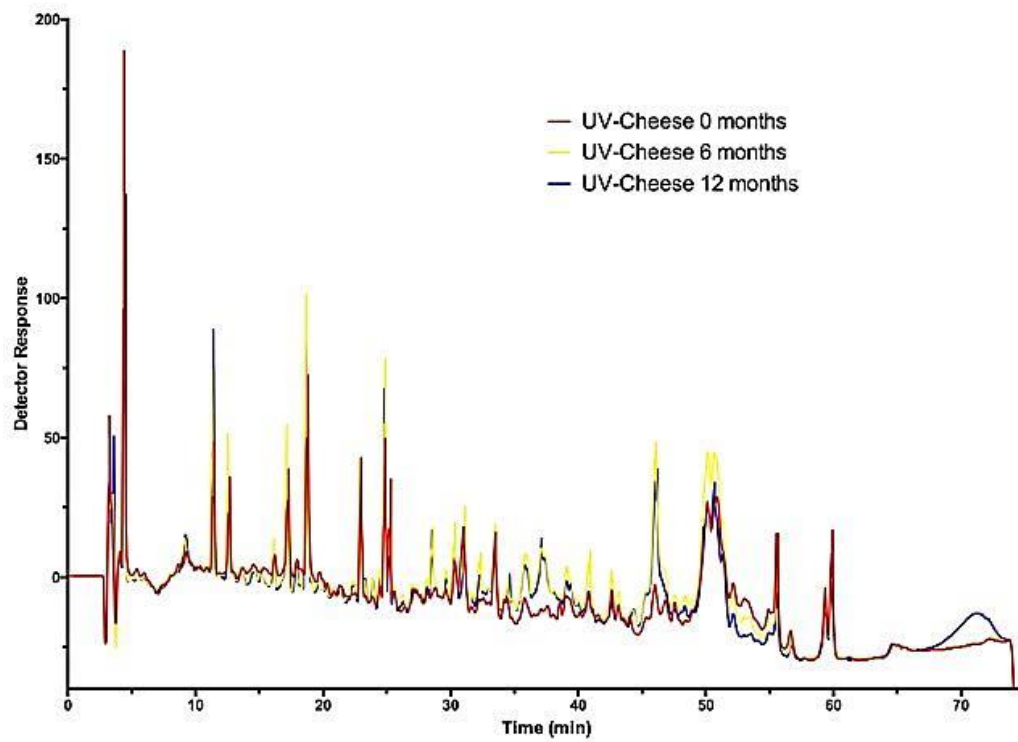


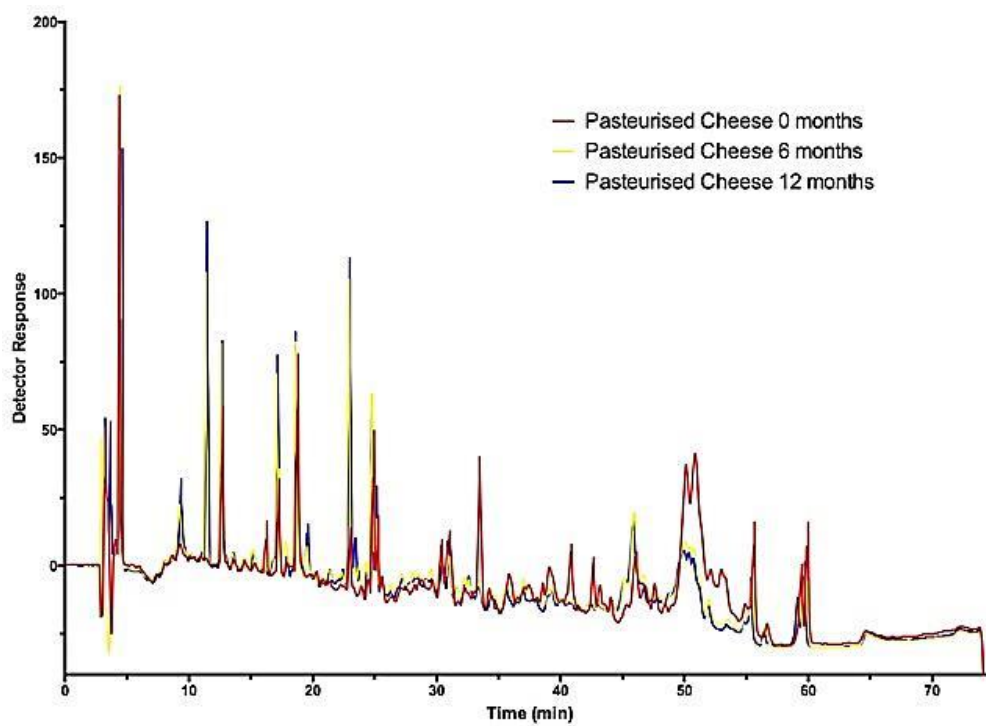
Figure 6.6a & b: Urea-PAGE of the WSN fraction (a) and WISN (b) in Cheddar cheese during different stages of ripening. Lane 1- P₀; Lane 2; P₆; Lane 3 – P₁₂; Lane 4; UVP₀; Lane 5 – UVP₆; Lane 6 – UVP₁₂; Lane 7 – UV₀; Lane 8 – UV₆; Lane 9 – UV₁₂.

RP-HPLC has found increased application in the characterization of peptides formed as a result of protein hydrolysis (Bicon, 1983). As cheese ripening progresses, the HPLC patterns become more complex and is often used to assess and the extent of proteolysis and ultimately final cheese quality (Fox, 1993). Analysis of the WSN fractions of the different Cheddar cheese treatments are indicated in Figure 6.7a (P), 6.7b (UV) and 6.7c (UVP). All three treatments displayed the development of new components during ripening of 12 months, characterized by peaks emerging at retention times between 11.5 and 25 minutes. The peaks represent the hydrophilic short peptides being formed as a result of proteolysis. The most significant peak development was recorded on all variants at 6 and 12 months of ripening, at the following retention times: 11.5, 13, 17, 19, 23 and 25 minutes (Figure 6.7). The most significant increase in peak development was observed at a retention time of 23 minutes for the P₆, P₁₂, UVP₆, and UVP₁₂ fractions. In contrast, the identical peak for the UV₆ and UV₁₂ samples was of lesser intensity than that for the P and UVP treatments respectively. Furthermore a more hydrophobic component, absent on day 0, eluting at 48 minutes, formed after 6 and 12 months ripening in the P and UVP treatments, with a concomitant reduction in the compound eluting at 52 minutes. However, the reduction of the peak at 52 minutes did not exhibit the same reduction pattern in the UV variant as for the P and UVP variants and remained constant throughout ripening. The profiles of the RP-HPLC chromatograms obtained from the cheese preparations in this study, compares well to previous RP-HPLC investigations of Cheddar cheese (Law *et al.*, 1992).

(a) UV



(b) P



(c) UVP

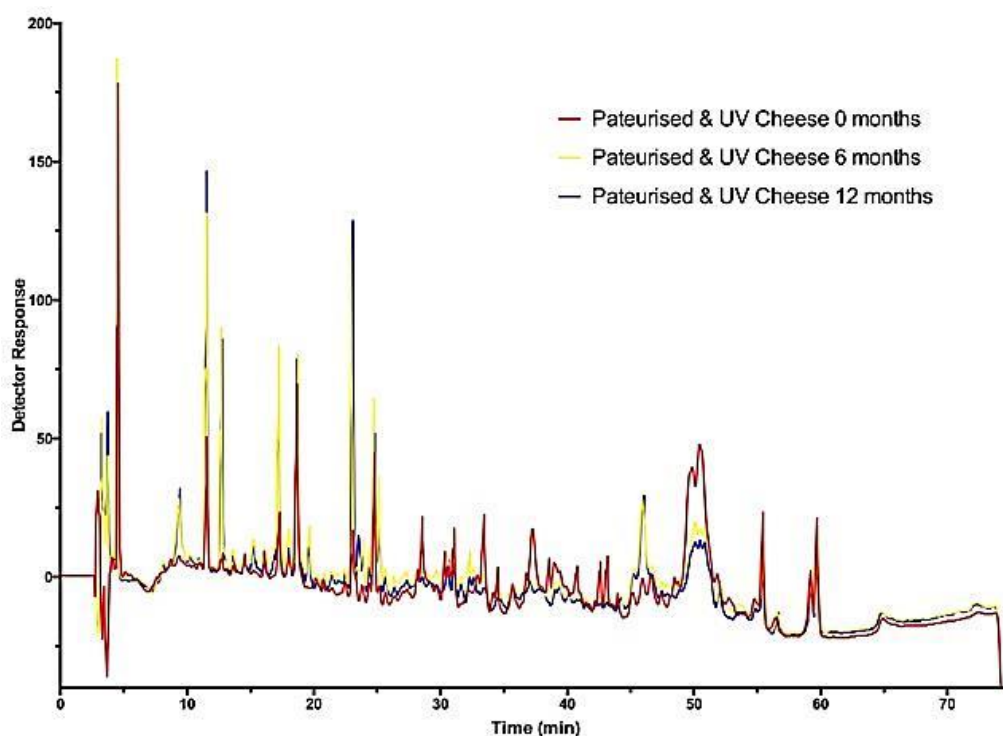


Figure 6.7: RP-HPLC chromatograms of the WSN of Cheddar cheese manufactured from (a) pasteurised milk (P) (b) UV milk (UV) and (c) UV and pasteurised milk (UVP) at day 0, after 6 months and 12 months of ripening.

6.4.3.4. *Effect of thermal pasteurisation and UV light treatments on the organoleptic quality of Cheddar cheese*

Samples of Cheddar cheese were evaluated for appearance, body and texture and flavour, to assess the extent of lipolysis and proteolysis on the day of production, after 8 weeks, 6 months and after 12 months of ripening. The results of the sensory analysis are summarized in Table 6.3. Sensory analysis was conducted on the P, UV and UVP Cheddar cheese samples during ripening. In this instance Cheddar cheese made of pasteurised milk (P) was considered as the control.

At the day of production and after pressing, the Cheddar cheese of all treatments was typical of a fresh cheese as far as appearance, body and texture and flavour, was concerned. No off-flavours were noted in any of the variants. The Cheddar cheese

was also evaluated after 2 months and results conformed to a typical young (green) Cheddar cheese on all the variants. A slight 'burnt' note identified in the UV₂ variant from some respondents, also present in the UVP₂ variant, although less pronounced ($p < 0.05$). The body and texture of the UV₂ cheese, when compared to the P₂ and UVP₂, rated slightly higher with the P₂ and UVP₂ samples having a shorter body and texture. Clean acid notes were present in all the variants, however; overall the flavour was not well developed in any of the cheeses evaluated.

Table 6.3: Sensory scores of Cheddar cheese manufactured from P, UV and UVP treated milk after 2 months, 6 months and 12 months.

Sample	Appearance (2)	Body and texture (7)	Flavour (11)	Score (20)
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
P ₂	1.6 \pm 0.3	3.8 \pm 0.8	5.9 \pm 1.0	11.3 \pm 1.4
UV ₂	1.3 \pm 0.4	3.9 \pm 0.6	5.6 \pm 1.2	10.7 \pm 1.4
UVP ₂	1.6 \pm 0.4	3.6 \pm 0.6	6.2 \pm 1.1	11.4 \pm 1.4
P ₆	1.6 \pm 0.4	4.7 \pm 0.4	7.4 \pm 0.7	13.8 \pm 1.1
UV ₆	1.3 \pm 0.3	4.5 \pm 0.8	7.8 \pm 1.3	13.6 \pm 1.2
UVP ₆	1.5 \pm 0.4	4.8 \pm 0.3	6.5 \pm 1.6	12.8 \pm 1.9
P ₁₂	1.5 \pm 0.4	4.3 \pm 0.3	6.7 \pm 1.7	12.5 \pm 1.6
UV ₁₂	1.4 \pm 0.4	5.9 \pm 0.6	8.9 \pm 0.7	16.3 \pm 1.6
UVP ₁₂	1.4 \pm 0.4	4.5 \pm 0.5	7.0 \pm 1.0	12.9 \pm 1.3

At 6 months cheese ripening period - sensory analysis reported flavours typical of a Cheddar cheese, with a slight 'burnt' note identified in the UV₆ variant from some respondents, also present in the UVP₆ variant, although less pronounced ($p < 0.05$). A slight bitter flavour was identified in all cheese samples, although not indicated as significant ($p > 0.05$). When compared to the P₆ and UVP₆ variants, the UVP₆ appearance had some salt-spots, which could have resulted from an uneven salt distribution or insufficient stirring of the curd during manufacture.

After 12 months - all the Cheddar cheeses evaluated were graded as typical Cheddar; with the 'burnt'-note in the UV₁₂ treated variants reduced significantly. The UV₁₂

sample exhibit 'meaty' notes which is agreed as positive and typical of SA Cheddar. Some of the panellists also picked up a 'smoked' flavour in the UV₁₂ and UVP₁₂ variants, which were graded as positive. The maturity and flavour development of the UV₁₂ overall was rated higher than that of the UVP₁₂ and P₁₂ Cheddar cheese. A definite and noticeable bitter flavour was identified in the P₁₂ and UVP₁₂ samples ($p < 0.05$), which was more pronounced when compared to the UV₁₂ samples, and did negatively impact on the scores of the UVP₁₂ and P₁₂ Cheddar cheese.

6.5. Conclusion

Three different treatments, heat pasteurisation, non-thermal turbulent flow UV treatment and combination of heat pasteurisation and UV treatment, were applied to raw milk before manufacturing of Cheddar cheese on a commercial scale. Minor differences in the fatty acid composition was noted between the different treatments, however, no correlation to flavour defects, such as rancidity or oxidative rancidity, could be established. Furthermore the free fatty acid values were very similar in all three cheese variants at any given time interval, and correlated well with the values reported in literature. It is therefore concluded that Cheddar cheese flavour development associated with lipolysis is more dependent on the formation of secondary metabolites produced through catabolism of free fatty acids, rather than the final concentration of free fatty acids in the Cheddar cheese itself. In general proteolysis and amino acid formation in Cheddar cheese has been found to correlate well with flavour and texture development during ripening, although the exact role of amino acids in flavour is not fully understood. Previous reports confirm that, although amino acids are not responsible for flavour development directly, it acts as important precursors for the formation of secondary flavour compounds through various enzymatic and non-enzymatic reactions (Engels and Visser, 1994). Even though a higher degree of proteolysis was expected in the UV cheese, the analytical results showed that the production of shorter peptides and α -amino-acids were lower than that of the P and UVP treated variants, most noticeable after 12 months of ripening. These results are consistent with findings of Andrews (1983) whom reported that the rate of casein hydrolysis, mainly β -casein, was greater in pasteurised milk than in raw milk.

Sensory analysis during the ripening of the Cheddar cheese indicated no major differences as far as the organoleptic properties were concerned, although the Cheddar cheese produced from milk treated with UV light alone was preferred to the P and UVP Cheddar after 12 months of ripening.

In conclusion, treatment of raw milk with UV light demonstrated immense promise as a novel technology that can be used for the production of safe, value added dairy products of good quality, such as Cheddar cheese. The use of UV as an alternative to pasteurisation in the study produced Cheddar cheese with a preferred organoleptic profile after 12 months. Although an increase in cheese yield was observed with milk treated with UV compared to the P and UVP variants, it should be the subject of further investigation to qualify. Particular areas of interest for future research would be the qualification of enzymatic action of different proteases (such as chymosin, indigenous milk proteinases and starter proteinases) responsible for the proteolysis following UV treatment of milk, and the qualification of secondary metabolites formed following proteolysis and lipolysis during ripening.

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Chapter 7

General summary and conclusions

The investigation of alternative processing technologies for the preservation of food and highly perishable liquids, such as fresh milk, remains an attractive and promising field of study. During the past two decades various research has been published on the efficacy of alternative processing technologies in the food industry, such as high pressure processing, electron and gamma irradiation, microwave processing, pulsed electric field, pulsed ultraviolet light and ultraviolet light processing.

The research conducted in this study focused on the use of ultraviolet light (UV) as an alternative processing technology to conventional heat pasteurisation in fresh milk. Critical control parameters (CCP), such as UV-C dose, flow-rate and the UV-dose response together with the design features of the SurePure TurbulatorTM were evaluated not only on pilot scale, but also within a commercial environment in order to determine the optimal CCP's for effective operation. The inactivation of microorganisms in fresh milk and the effect of UV-treatment on the organoleptic and physio-chemical characteristics in milk and Cheddar cheese manufactured from UV-treated milk were investigated.

This is the first time that a comprehensive study has been carried out to investigate the effect of ultraviolet treatment on fresh milk and Cheddar cheese manufactured from UV-treated milk within a commercial environment, thus affording valuable insight into the potential translation of UV technology into industrial application. The UV technology, either as a single stand-alone technology or as a complementary step with thermal processing can produce high quality secondary dairy products, such as Cheddar cheese, as was concluded in this study. The contributions to scientific knowledge can be summarized as follows:

1. The inactivation kinetics and UV-dose responses of aerobic plate counts, coliforms, *E.coli* and psychrotrophic bacteria in fresh and inoculated whole milk with UV treatment was modelled;

2. An alternate treatment method to characterize the amount of UV energy delivered per volume of milk treated in turbulent, thin-film continuous-flow processing UV-equipment was suggested;
3. The effect of UV-treatment on fresh milk, with specific mention to fat and protein oxidation, lipolysis, proteolysis, organoleptic properties and the chemical composition was qualified and quantified following UV-treatment;
4. For the first time, proteolysis and lipolysis in commercial processing of Cheddar cheese curd produced from UV treated milk was determined and compared with those from thermally pasteurised milk.

In regards to future studies within the field of UV-treatment of milk, the following research should be considered:

1. The investigation into alternative sources of UV-light with enhanced light emission properties to be used within turbulent, thin-film continuous-flow processing UV-equipment, such as light-emitting diodes (LED's). The isolation of the most germicidal wavelengths from the UV-C spectrum could be advantageous in limiting possible defects such as oxidation of protein in milk due to the over-exposure of UV-light from conventional UV-light sources when treating liquids with a low UV transmission;
2. The most UV-resistant pathogen should be isolated and UV-dose responses should be quantified in order to limit the risk associated with UV-treatment within commercial application. Furthermore the possible investigation into 'hurdle' technology should be considered, using a combination of alternative processing technologies, including UV-treatment, in order to enhance food safety and germicidal efficacy;

3. The qualification of enzymatic action of different proteases (such as chymosin, indigenous milk proteinases and starter proteinases) responsible for the proteolysis following UV treatment of milk, and the qualification of secondary metabolites formed following proteolysis and lipolysis during ripening.

In summary, UV-technology in future could provide to be a viable alternative to conventional thermal processing within the dairy industry, especially in lieu of the recent advances in regards to non-thermal processing techniques, equipment and methodology. Research within this technology area could positively contribute to limit the environmental impact of current thermal processing methods, deliver future sustainability in the areas of food production and food protection and deliver growth in developing and developed markets within the general food and dairy industries.

ANNEXURES

Annexure A



US006916452B1

(12) **United States Patent**
Rix et al.

(10) **Patent No.:** **US 6,916,452 B1**
(45) **Date of Patent:** **Jul. 12, 2005**

(54) **STERILIZATION OF LIQUIDS USING
ULTRA-VIOLET LIGHT**

(75) Inventors: **Eldred Rix, Kirstenhof (ZA); Attila
Kurucz, Goodwood (ZA)**

(73) Assignee: **Hydrozone, Ltd.**

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 23 days.

(21) Appl. No.: **10/110,448**

(22) PCT Filed: **Oct. 12, 2000**

(86) PCT No.: **PCT/ZA00/00189**

§ 371 (c)(1),
(2), (4) Date: **Aug. 12, 2002**

(87) PCT Pub. No.: **WO01/37675**

PCT Pub. Date: **May 31, 2001**

(30) **Foreign Application Priority Data**

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Jan. 18, 2000 (ZA) 2000/0188

(51) Int. Cl.⁷ **C02F 1/32**

(52) U.S. Cl. **422/186.3; 210/748; 250/438**

(58) Field of Search 210/748, 198.1,
210/205; 422/24, 186.3; 250/432 R, 435,
250/436, 437, 438

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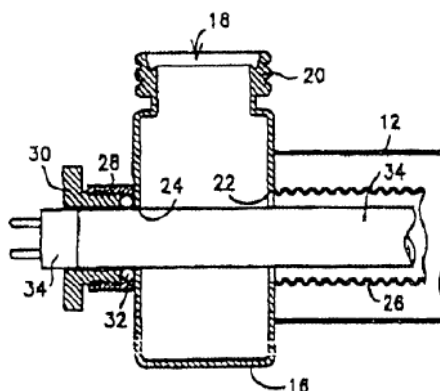
Primary Examiner—Frank M. Lawrence

(74) *Attorney, Agent, or Firm*—J. Wiley Horton

(57) **ABSTRACT**

A sterilizer for milk and other liquids is disclosed, the sterilizer having an elongate outer housing (12) with a manifold (16) at each end. Dairy fittings (20) forming the inlet to and outlet from the sterilizer protrude from the manifolds (16).

3 Claims, 2 Drawing Sheets



U.S. Patent

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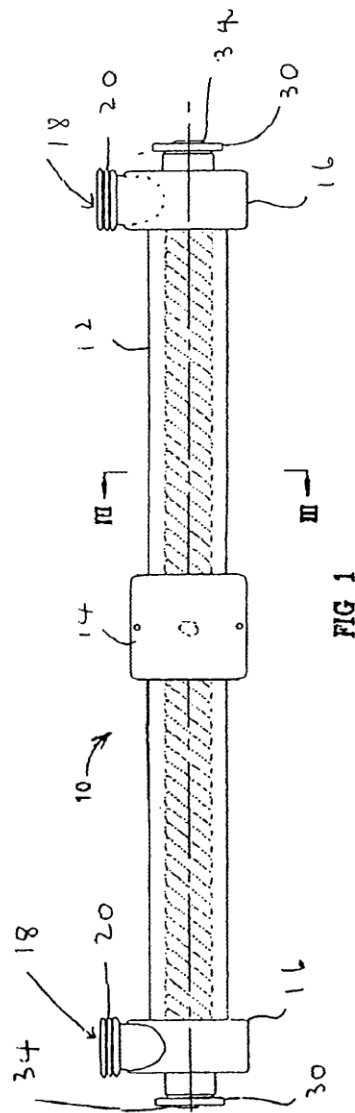


FIG 1

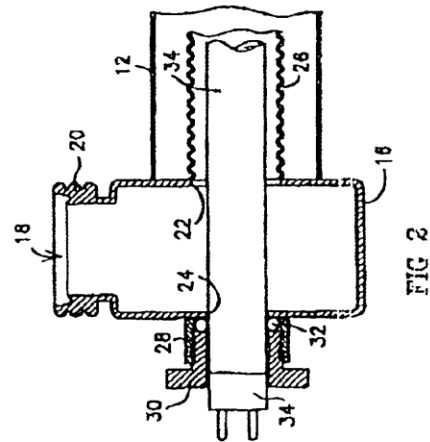


FIG 2

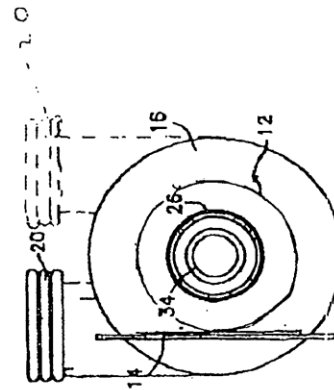
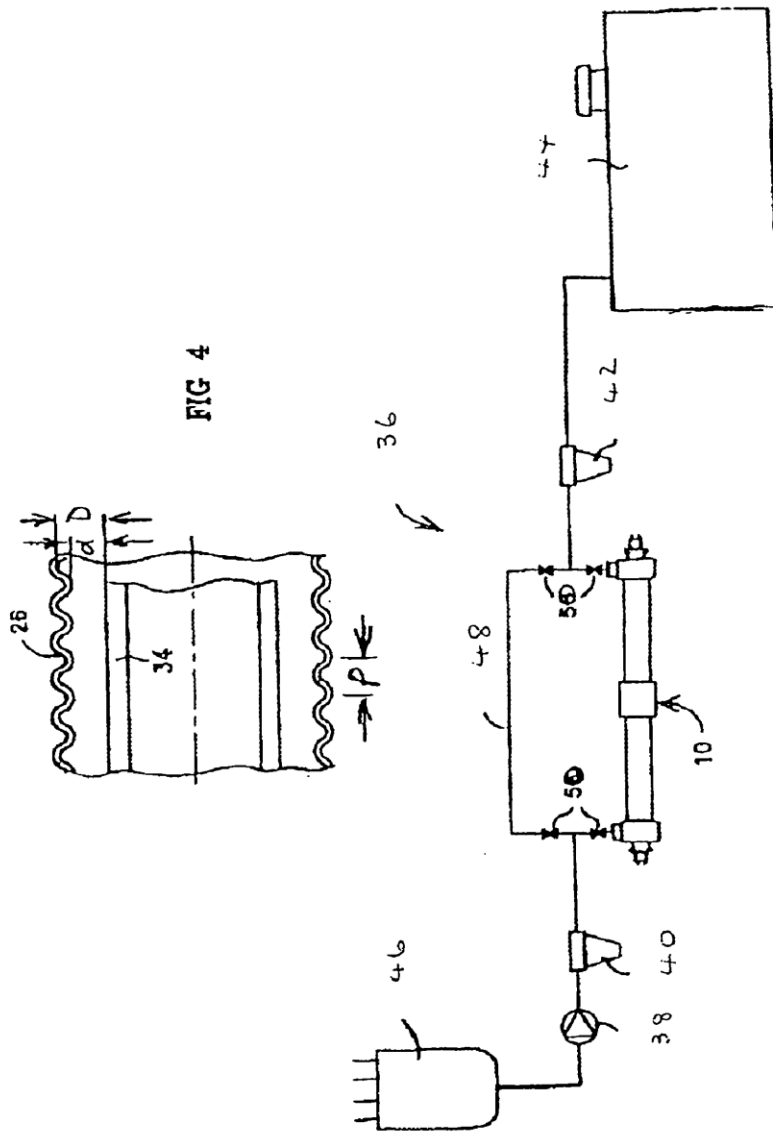


FIG 3



US 6,916,452 B1

1 STERILIZATION OF LIQUIDS USING ULTRA-VIOLET LIGHT

FIELD OF THE INVENTION

THIS INVENTION relates to the sterilization of liquids using ultra-violet light.

The use in this specification of the term "sterilization" is meant to indicate a reduction in bacterial count in a liquid, and not necessarily a total elimination of bacteria.

BACKGROUND TO THE INVENTION

The use of ultraviolet (UV) light for the purpose of sterilizing a liquid is well known. A problem that arises with a turbid liquid is that the light does not penetrate very far into the liquid and hence liquid furthest from the UV lamp may not be sterilized at all or may not be properly sterilized.

South African patent specification 96/8029 discloses an elongate sterilizer in which a fluorescent tube is within, and co-axial with, an elongate housing. The sterilization chamber is between the fluorescent tube and the housing. The liquid inlet and liquid outlet are arranged tangentially with respect to the housing in an effort to cause the liquid to swirl and overcome the difficulty referred to above. It has been found, however, that the swirling motion imparted to the liquid as it enters the housing does not continue throughout the length of the housing, thus limiting the beneficial effect.

Other structures are known, such as that shown in U.S. Pat. No. 5,675,153, in which there is a helical vane in the space between the fluorescent tube and the housing, the vane extending from one end of the housing to the other. The vane is slotted and there is a gap between the vane and the inner surface of the housing. Such a structure would be completely unsuitable for the sterilization of milk, because of the many sharp corners where flow would stagnate and bacteria would be able to multiply.

There is a tendency for solids in milk to deposit on the surfaces defining the flow passage, in regions where there is insufficient flow velocity, so that the structure of U.S. Pat. No. 5,675,153 if it were to be used for the sterilization of milk, would suffer from deposits and as a consequence would require frequent cleaning. The structure would, in any event, be difficult to clean to the degree that is required in apparatus that is used for the handling of milk.

BRIEF DESCRIPTION OF THE INVENTION

According to one aspect of the invention there is provided a sterilizer for reducing the bacteria count in a liquid, the sterilizer comprising an elongate sheath, an elongate fluorescent tube extending along the sheath, there being a gap between the tube and the sheath through which gap the liquid to be sterilized flows, said sheath having an internal configuration including protuberances over which, in use, the liquid flows and which impart turbulence to the flowing liquid, and an inlet for the liquid which inlet is offset with respect to the sheath and the tube so as to cause the incoming liquid to swirl in the sheath.

In one form the sheath has a spiral groove in the inner face thereof with a spiral land between adjacent turns of the groove, the land forming said protuberances over which the liquid being sterilized flows. The shape of the protuberances is preferably such that they provide the inner surface of the sheath with a smoothly curved, undulating configuration. Preferably said tube and sheath are within and extend along an elongate outer housing.

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According to a further aspect of the present invention there is provided a method of reducing the bacteria count in milk, the method comprising causing the milk to flow from a milking machine to a sterilizer in which the milk is subjected to ultraviolet radiation, the milk being subjected to said ultraviolet radiation before it cools to below 28° C.

The method can include the further step of cooling the milk down to storage temperature after subjecting it to ultraviolet radiation.

BRIEF DESCRIPTION OF THE DRAWINGS

For a better understanding of the present invention, and to show how the same may be carried into effect, reference will now be made, by way of example, to the accompanying drawings in which;—

FIG. 1 is a side elevation of a sterilizer in accordance with the present invention;

FIG. 2 is a longitudinal section through one end of the sterilizer, drawn to a larger scale;

FIG. 3 is a cross-section on line III—III of FIG. 1, drawn to the same larger scale;

FIG. 4 is a detail of part of FIG. 2; and

FIG. 5 is schematic diagram of a milk sterilization installation in accordance with the invention.

DETAILED DESCRIPTION OF THE DRAWINGS

Referring firstly to FIGS. 1 to 4, the sterilizer 10 illustrated is designed specifically for the purpose of sterilizing milk but can be used to sterilize not only turbid liquids but also transparent or translucent liquids. The sterilizer comprises an elongate stainless steel outer housing 12 which is circular in cross section. A mounting plate 14 is tack welded to the housing 12 midway between its ends (see FIG. 3). In another form the outer housing 12 is square in cross section.

At each end of the housing 12 there is an manifold 16, one manifold forming an inlet for milk to be sterilized, and the other manifold forming an outlet for sterilized milk. The manifolds 16 each have a port 18. The ports are each in a conventional mate dairy fitting 20 whereby a hose can be attached thereto. The fitting 20 of one manifold 16 is to one side of the vertical centre plane of the sterilizer (as shown in full lines in FIG. 3) and the fitting 20 of the other manifold 16 is to the other side of the centre plane as shown in dotted lines in FIG. 3.

Each manifold 16 has aligned openings 22, 24 in opposite walls thereof. The sterilizer further comprises a corrugated sheath 26 which is aligned with the opening 22 and extends the full length of the housing 12 between the manifolds 16.

Each manifold 16 has an internally threaded socket 28 secured thereto, the sockets 28 being aligned with the openings 24.

An externally threaded bush 30 is screwed into each socket 28 and there is a sealing ring 32 between each bush 30 and the wall of the manifold.

A fluorescent tube 34 (also referred to as a germicidal UV lamp) passes through the bushes 30, sockets 28, sealing rings 32, manifolds 16 and sheath 26, the ends of the fluorescent tube protruding from the bushes 30. When the bushes 30 are tightened the sealing rings 32 are compressed and grip the fluorescent tube 34, thereby forming liquid-tight seals.

The sheath 26 is of stainless steel and is formed with a helically extending corrugation. As can best be seen in FIG. 4, the helical corrugation has a pitch P of about 6 mm and provides the inside of the tube with a smoothly curved

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undulating surface when the tube is viewed in radial cross section, without any sharp corners or discontinuities where flow stagnation can occur.

The sheath **26** has a nominal diameter of about 40 mm. Being of stainless steel, the inner surface of the sheath **26** is reflective. The radial gap between the fluorescent tube **34** and the sheath **26** varies between about 5 mm at the troughs of the corrugations (the distance d) to about 7 mm at the crests (the distance D).

In another form the sheath **26** has a spiral groove extending along the inner face thereof with a spiral land separating adjacent turns of the groove. The gap between the land and the outer face of the tube **34** is approximately 5 mm.

Milk flowing through the sterilizer **10** passes through the narrow annular gap between the fluorescent tube **34** and the corrugated sheath **26**. As the milk flows into the sterilizer a swirling motion is imparted to it, and hence turbulence is introduced, by the tangential position of the inlet fitting **20**. The corrugations maintain, throughout the length of the sterilizer, the turbulence introduced into the milk as it flows into the manifold. This ensures that all the milk is subjected to UV light. The tangential arrangement of the outlet fitting **20** ensures that the milk flows smoothly out of the sterilizer without the fitting causing a back pressure which could dampen the turbulent flow.

If the inlet manifold and fitting do not impart turbulence to the incoming liquid, then the surface of the sheath breaks up the smooth flow of the incoming liquid and introduces turbulence.

It has been found that the best results are obtained when the velocity of the milk flowing through the gap between the fluorescent tube **34** and the sheath **26** is about 3 m/s, preferably between 3 and 3.5 m/s. At lower flow velocities there is a fall off in the turbulence that is required to ensure a proper irradiation of all the milk. At higher flow velocities, there is a tendency for butter formation to take place. There is also at higher velocity a tendency for the tube **34** to be coated thereby blocking off UV light.

Referring now to FIG. 5, reference numeral **36** generally indicates an installation for sterilizing milk, the installation being erected on a dairy farm and including a sterilizer **10** of the type described above with reference to FIGS. 1 to 4.

The installation **36** comprises a pump **38**, a first filter **40** connected upstream of the sterilizer **10**, a second filter **42** connected downstream of the sterilizer **10**, and a bulk cooler tank **44**. The pump **38** has its suction inlet connected to the milk collecting bowl **46** of a milking machine, and pumps the milk from the milk collecting bowl to the bulk cooler tank **44** via the first filter **40**, the sterilizer **10** and the second filter **42**. Cooling of the milk takes place in the tank **44**.

The installation **36** includes a bypass line **48** bypassing the sterilizer **10**. Bypass valves **50** are provided to divert flow from the sterilizer **10** to the bypass line and to isolate the sterilizer. If desired, the bypass line **48** may be substituted by a second sterilizer **10** so that flow can be diverted from one sterilizer to the other.

The filter **40** is provided to filter out hair and other dirt from the milk that is received from the milk collecting bowl. The second filter **42** is provided as a safety feature, to

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prevent glass fragments or other parts of the fluorescent tube **34** from finding their way into the tank **44** in the event of a breakage.

Two or more sterilizers **10** can be provided in series.

It is an important feature of the invention that the milk is subjected to ultraviolet radiation in the sterilizer **10** while the milk is still warm. The fatty constituents of milk start to separate from the rest of the milk when the temperature falls below 28° C. This is referred to as "crystallization". By passing the milk through the sterilizer while the milk is still at a temperature of 28° C. or above the tendency of the fatty constituents to collect on the inside surfaces of the sterilizer is minimized. Thus sterilization occurs before the milk cools to below 28° C.

While the use of the sterilizer **10** to irradiate milk has been described above it is to be understood that the sterilizer could also be used to sterilize other liquids. For example, it could be used to sterilize liquids such as wine and petroleum.

What is claimed is:

1. A sterilizer for reducing the bacteria count in a liquid, comprising:

- a. an elongate sheath, having a first end, a second end, and a central axis;
- b. an elongate fluorescent tube extending along said sheath, there being a gap between said tube and said sheath through which said liquid flows;
- c. wherein said sheath has a surface facing toward said fluorescent tube, wherein said surface includes protuberances over which, in use, said liquid flows, thereby imparting turbulence to said flowing liquid;
- d. wherein said surface facing toward said fluorescent tube has a smoothly curved spiral groove with a smoothly curved spiral land between adjacent turns of said smoothly curved spiral groove, with said smoothly curved groove and said smoothly curved land forming said protuberances over which said liquid flows;
- e. an inlet manifold, connected to said first end of said elongate sheet; and
- f. said inlet manifold including an inlet port for said liquid, wherein said inlet port is offset from said central axis of said elongate sheet in order to impart rotational flow to said liquid as said liquid flows into said first end of said elongate sheath.

2. A sterilizer as claimed in claim 1, wherein said tube and said sheath are within and extend along an elongate outer housing.

3. A sterilizer as claimed in claim 1, further comprising an inlet which is offset with respect to said sheath and said tube so as to cause said liquid to swirl as it flows into said sheath, an outlet manifold, connected to said second end of said elongate sheath, with said outlet manifold including an outlet port for said liquid, wherein said outlet port is offset from said central axis of said elongate sheath in order to maintain said rotational flow of said liquid as said liquid flows into said second end of said elongate sheath.

* * * * *

Annexure B



US008127667B2

(12) **United States Patent**
Kastenschmidt et al.

(10) **Patent No.:** **US 8,127,667 B2**
(45) **Date of Patent:** **Mar. 6, 2012**

(54) **APPARATUS FOR PASTEURIZING MILK FOR FEEDING TO CALVES**

(75) Inventors: **Kevin M. Kastenschmidt**, Rockland, WI (US); **Matthew J. Stuessel**, Alma Center, WI (US); **David A. Becker**, Winona, MN (US); **Robert L. Buck**, Holmen, WI (US); **Ralph A. Rottier**, Cable, WI (US)

(73) Assignee: **GEA Farm Technologies, Inc.**, Naperville, IL (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 391 days.

(21) Appl. No.: **12/383,830**

(22) Filed: **Mar. 27, 2009**

(65) **Prior Publication Data**
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(51) **Int. Cl.**
A23C 3/02 (2006.01)
A23C 3/07 (2006.01)
(52) **U.S. Cl.** **99/451**; 99/453
(58) **Field of Classification Search** 99/451, 99/453, 455, 483
See application file for complete search history.

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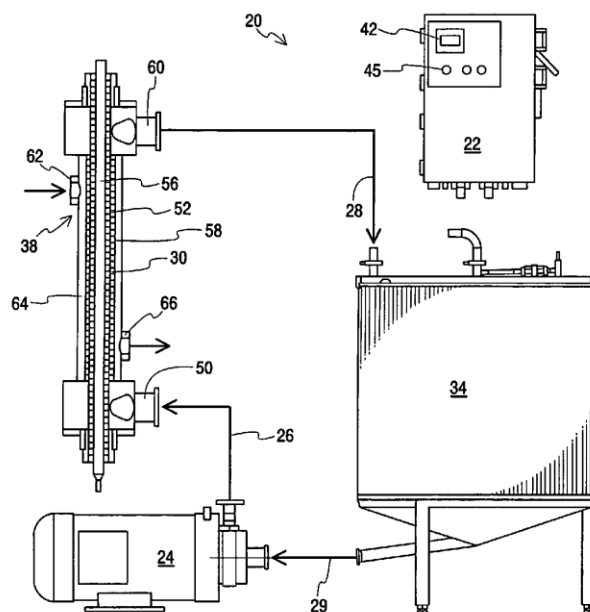
Primary Examiner — Stephen F Gerrity

(74) *Attorney, Agent, or Firm* — Smith Law Office

(57) **ABSTRACT**

The present invention is directed to a system and method for pasteurizing milk and colostrum for feeding to calves. The system includes a vat for storing milk or colostrum, a circulation pump and piping system, a heat exchanger for adjusting and/or maintaining milk temperature, and an ultraviolet light pasteurizing unit ("UV reactor") that treats the milk without damaging important immunoglobulins.

12 Claims, 7 Drawing Sheets



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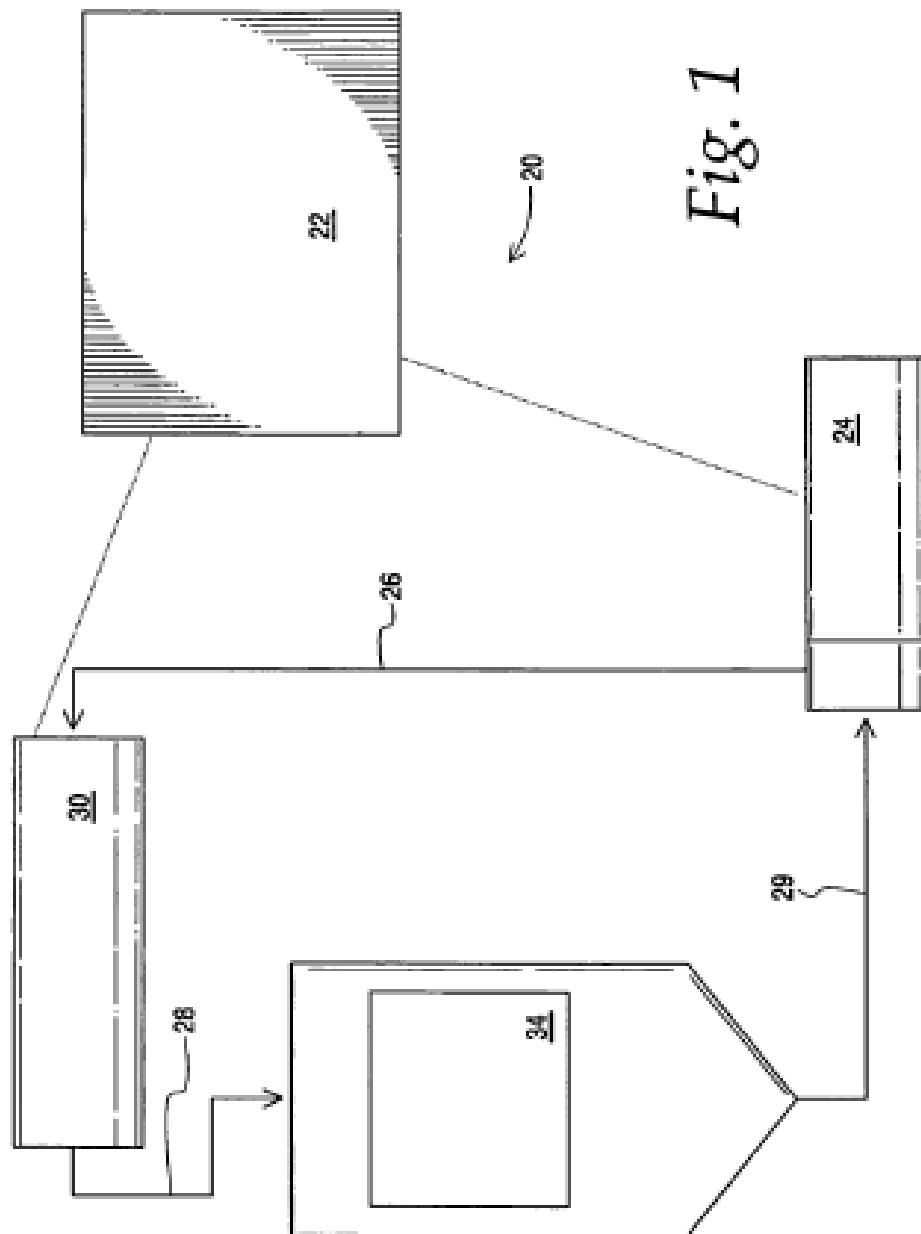
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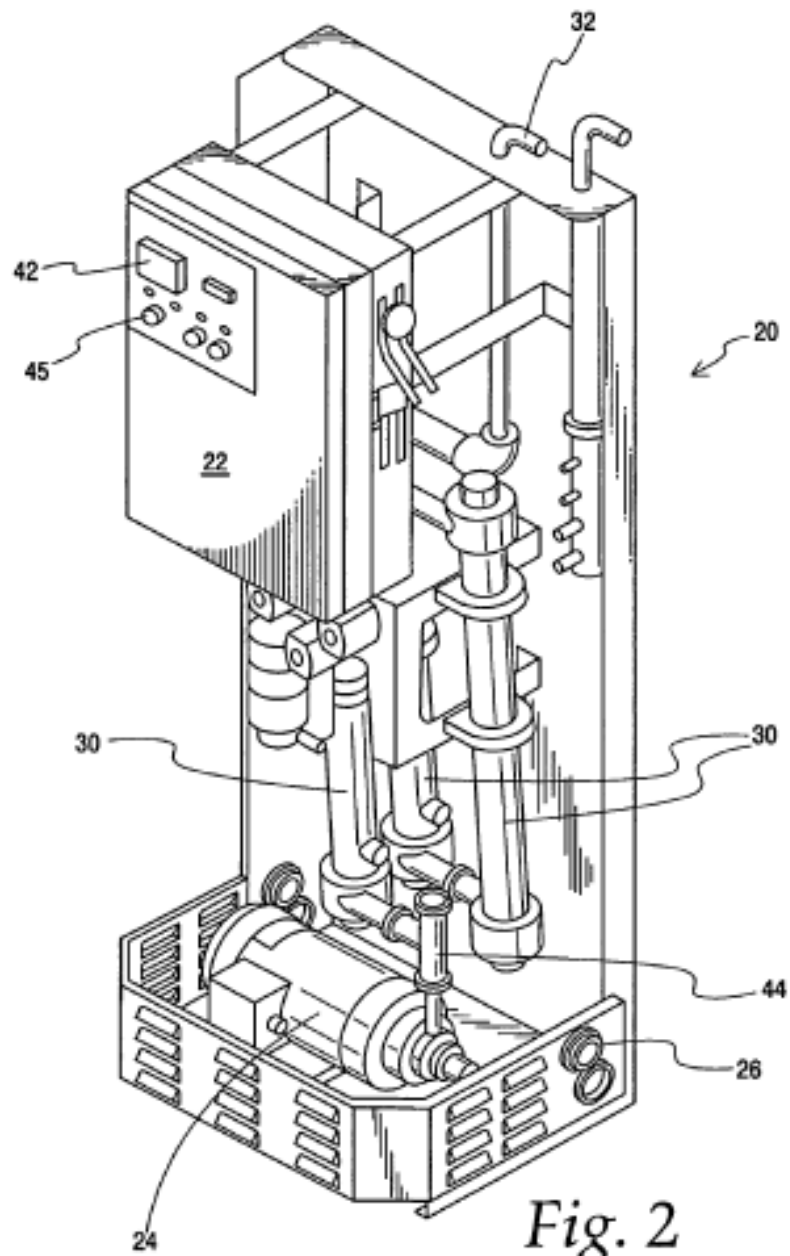
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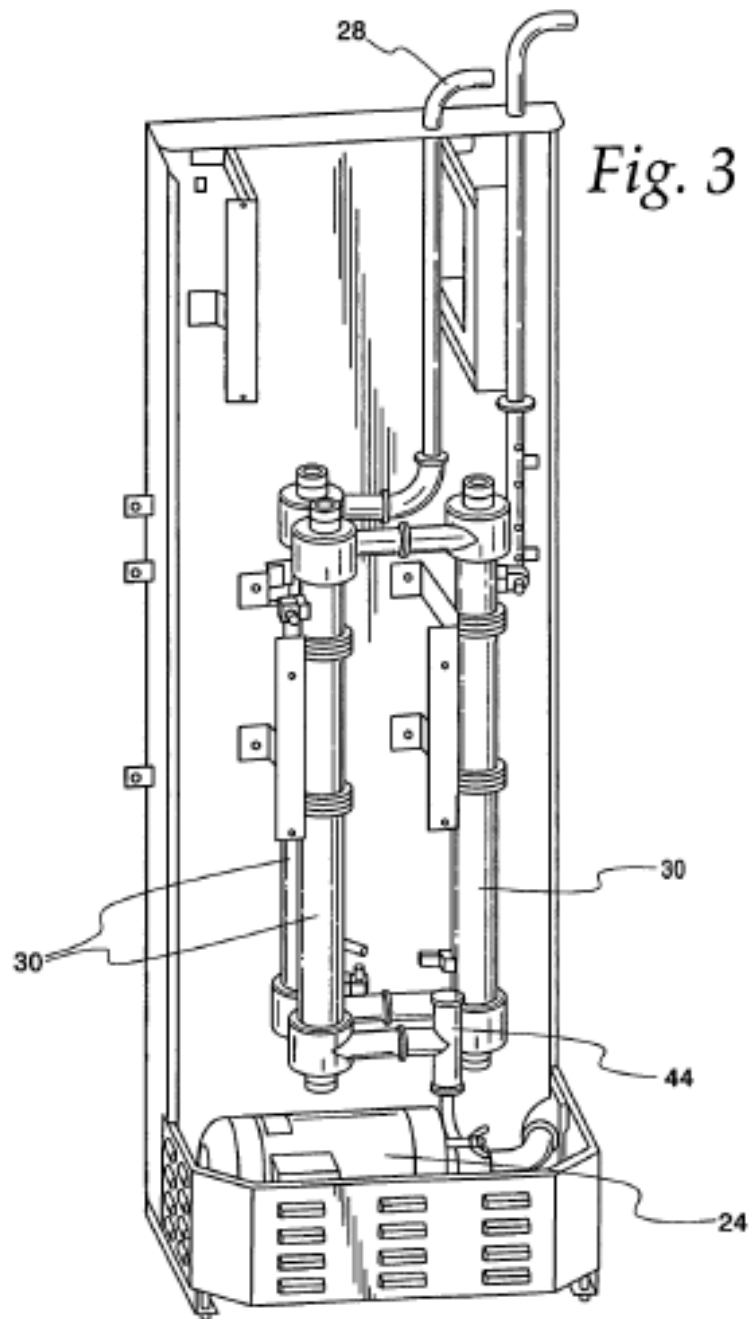
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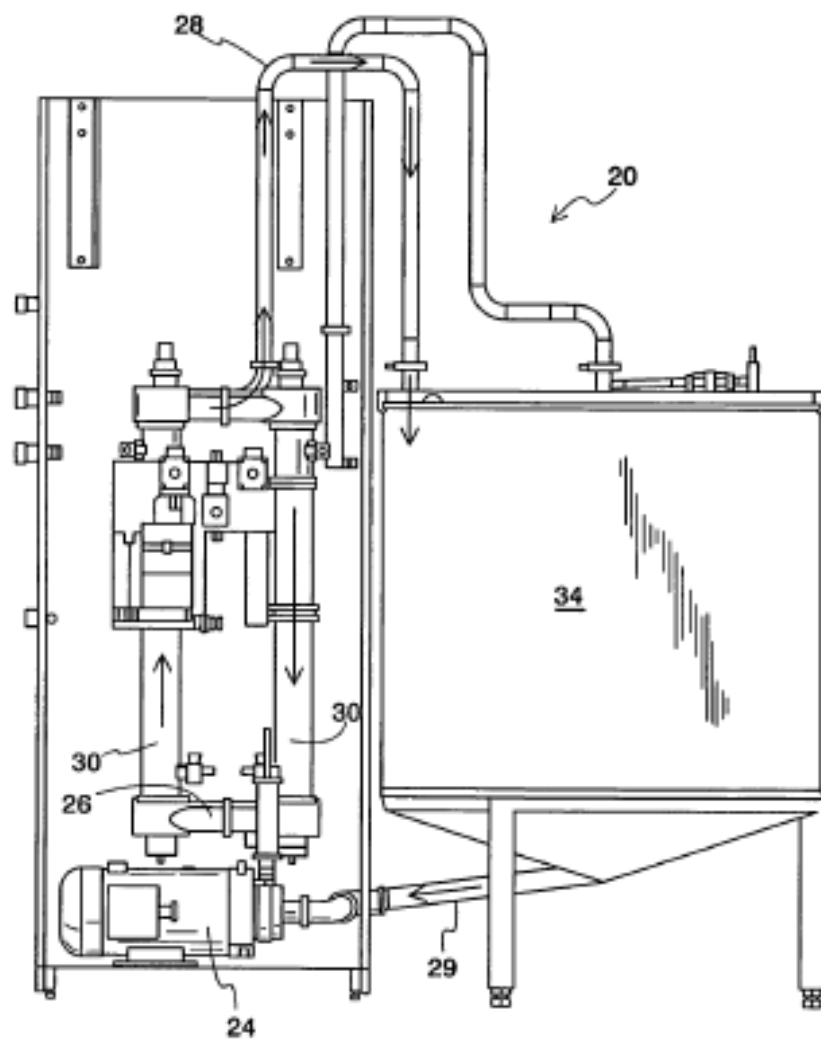
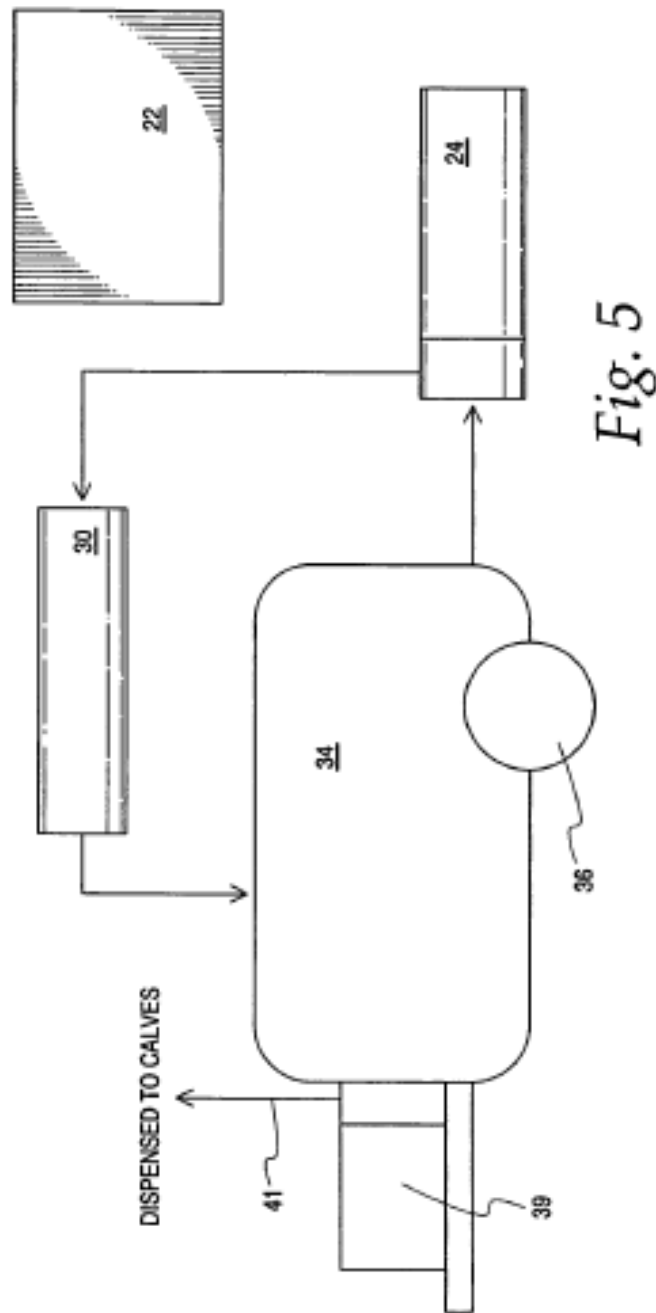


Fig. 4



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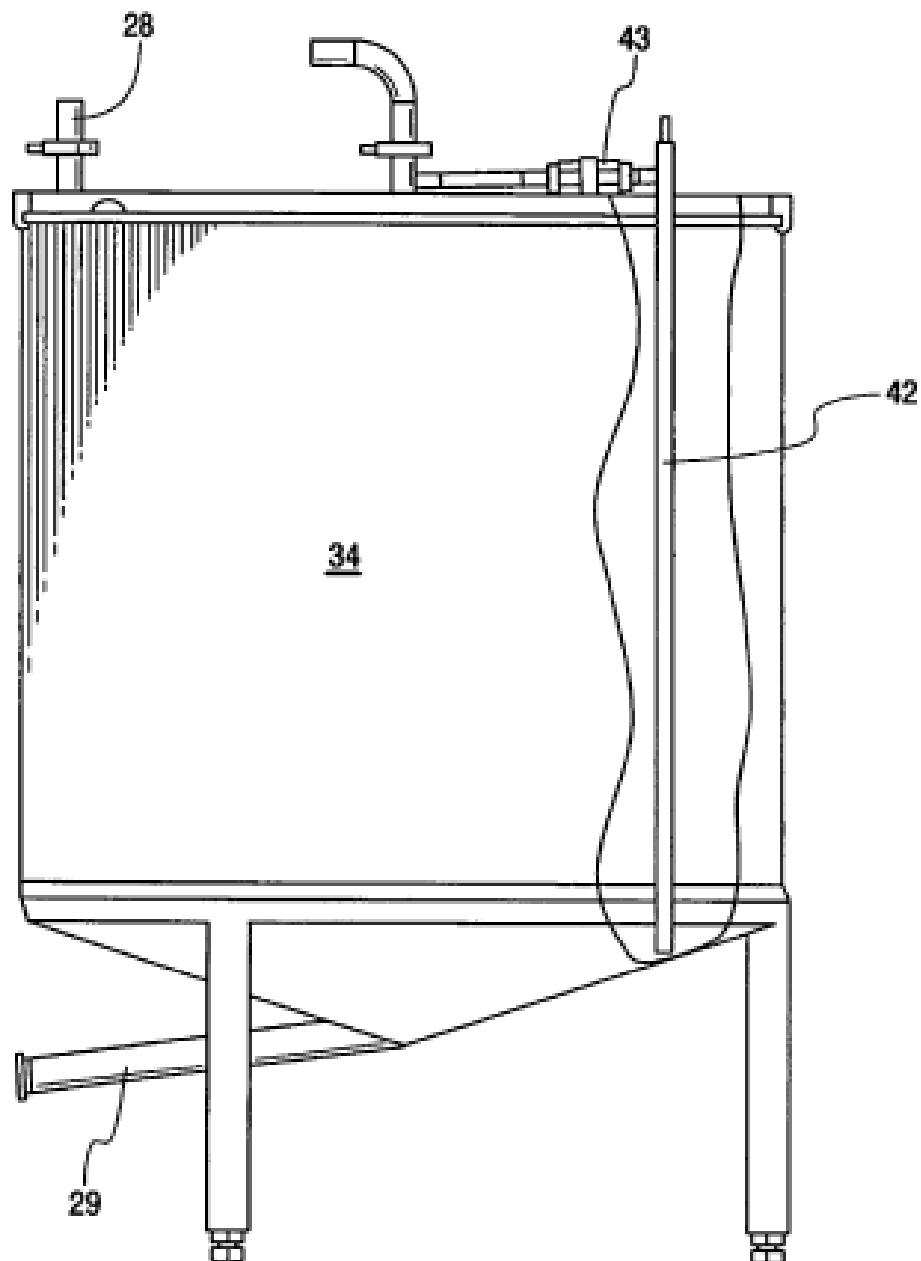
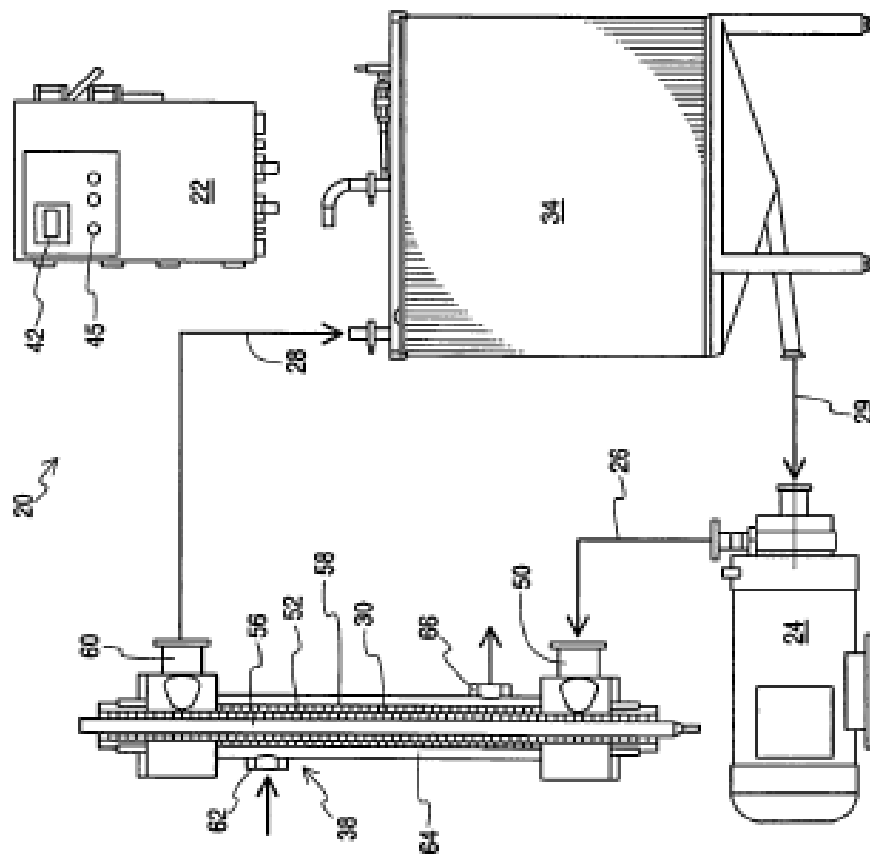


Fig. 6

Fig. 7



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**APPARATUS FOR PASTEURIZING MILK
FOR FEEDING TO CALVES****FIELD AND BACKGROUND OF THE
INVENTION**

This invention relates generally to liquid sterilization systems and more particularly to apparatus and methods for pasteurizing milk and colostrum for feeding to calves.

Calves in dairy harvesting facilities are fed non-saleable ("waste") milk collected from cows that have been removed from the main herd for medical or other reasons. While the feeding of non-saleable milk to dairy calves would seem to be economical for the dairy operator, there is a risk that infectious pathogens may be transmitted through the milk or shed directly from the cow's mammary gland. Other pathogens can be deposited in milk from manure or dirt, or can result from proliferation in milk that is not chilled or stored properly.

To reduce this risk, it is preferred that milk or colostrum be pasteurized before feeding to calves. Pasteurization in known processes includes heating milk to a target temperature to kill a target microbe and maintaining that temperature for a period of time. The pasteurized milk ordinance defines two different methods for pasteurization: 1) batch pasteurization at 145° F. for 30 minutes (low-temperature, long-time or LTLT), or 2) high-temperature, short-time pasteurization (HTST) at 161° F. for 15 seconds (usually using a continuous flow process). Heating and maintaining the heat above a target temperature results in a log reduction in concentration of viable bacteria. However, some heat-tolerant bacteria may survive the process. Further, in a poor quality milk with very high concentrations of bacteria, some pathogenic bacteria may survive the pasteurization process.

Pasteurization is desirable and sometimes necessary to kill bacteria, such as *E. coli*, *B. Cereus*, and *salmonella* that are harmful to calves. Heat pasteurization to 145° F. is successful at killing nearly 100% of these bacteria if the milk is maintained above 145° F. for at least thirty minutes. One study suggests that a lower temperature of 120° F. can be used, but that temperature must be maintained for at least sixty minutes.

Once pasteurized, milk may be bottled, chilled, stored, and then reheated to a feeding temperature of between 100° F. to 110° F. It may not be necessary to store pasteurized milk because it is more readily available and can be fed directly to calves after it is heat pasteurized and cooled to feeding temperature.

Also, colostrum is fed to newborn calves within two hours of birth and again within twelve hours of birth. Colostrum is collected from cows shortly after calving, and includes relatively high concentrations of carbohydrates, protein, and antibodies. Colostrum also contains high concentrations of immunoglobulins, such as IgG, and growth factors. Pasteurizing colostrum can result in congealing and loss of immunoglobulins. About 25% to 30% of IgG concentrations in colostrum are destroyed in heat pasteurization of colostrum and milk. Thus, heat pasteurization is beneficial overall, but has detrimental effects on milk and colostrum. Colostrum is usually chilled, bottled, and stored prior to re-heating and feeding.

Ultraviolet pasteurizers can also be used to treat milk. U.S. Pat. No. 6,916,452, to Rix et al. discloses that milk can be sterilized in a dairy using one or more UV sterilizer units while maintaining milk temperature above 82.4° F. (28° C.) before it is transferred to a chiller and a bulk milk storage vat. Such a pasteurizer is conceptually well-founded, but is not

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able to be used on its own in a dairy facility because it lacks critical features necessary to prepare the milk and colostrum for distribution and feeding to calves.

An improved pasteurization system is needed that successfully kills harmful bacteria, but destroys little or no immunoglobulins for optimum calf health.

SUMMARY OF THE INVENTION

The present invention is directed to a system and method for pasteurizing milk and/or colostrum for feeding to calves. The term "milk" as used herein should be understood to include milk, colostrum, other calf feed, and any related supplements. "Calves" as used herein includes any dairy animal such as cows, goats, and sheep.

The system includes a vat or vat for storing milk or colostrum, a circulation pump and piping system, a heat exchanger for adjusting and/or maintaining milk temperature, and an ultraviolet light pasteurizing unit ("UV reactor"). A controller activates the pump to circulate milk to a heat exchanger to raise milk temperature to between 85° F. and 120° F. and then circulates the warmed milk through the UV reactor at an appropriate rate and frequency to kill harmful bacteria. The milk can then be chilled and stored or fed directly to calves. By raising the milk temperature to only the range of 85° F. to 120° F., there is much less destruction of immunoglobulins and the milk is still safely pasteurized by the UV light from the UV reactor.

Unlike batch pasteurizers, UV reactors do not rely on the temperature of the milk to kill bacteria. Instead, the UV light alone in the range of 200 to 280 nanometers, UVC range (germicidal range), kills the bacteria. Nonetheless, milk temperature is important because cold milk is churned by the pump and piping system, and butter flakes can form that are less likely to be adequately treated by the UV light. Raising milk temperature to 85° F. or higher is sufficient to melt or reduce the size of butter flakes that form so that the milk is adequately treated by the UV reactors. Preferably, the milk temperature is raised to above 95° F., and more preferably to above 100° F. to ensure proper milk viscosity with minimal butter concentrations. On the other hand, heating milk to too high of a temperature can destroy beneficial immunoglobulins. An upper end of the temperature range to minimize destruction of the immunoglobulins is 120° F., and preferably 115° F., and more preferably 110° F.

A temperature range of about 85° F. to about 120° F. includes a feeding temperature range of between about 100° F. and about 110° F. If the milk is to be fed directly to calves, then heating to the feeding temperature range of between about 100° F. to 110° F. for pasteurizing is appropriate.

If the milk is to be chilled and stored after pasteurization, a milk temperature in the lower end of the range of 85° F. to 120° F. will produce adequate results with the present invention and reduce energy requirements. It is noted that treated waste milk may need to be transported to the calves where they are kept in the dairy facility. In this situation, heating the milk to above the feeding temperature range can compensate for milk cooling as it is being transported. Heat loss is a function of ambient conditions, the time between pasteurization and feeding, and other factors. Thus, using the present invention, milk temperature can be adjusted to compensate for these and other factors in any particular dairy situation.

Apparatus in accordance with the present invention can include one or more UV milk pasteurizer reactors, flow controllers, temperature controllers, and devices for setting and adjusting optimal milk temperature for milk leaving the appa-

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ratus to accommodate calf needs, temperature losses for time, distance and methods for transporting the milk to calves.

UV milk pasteurizers for use in the present invention can be those of the type disclosed in Rix et al., U.S. Pat. No. 6,916, 452 (incorporated herein by reference). A number of such pasteurizers can be used in series to reduce the number of times milk is circulated through the UV reactors.

Preferably, the controller of present invention monitors UV reactor components and adjusts treatment time to accommodate defective UV bulbs, ballasts, or related components.

Flow controllers for use in the present invention include pumps and meters that pump milk through the UV reactor at a rate that ensures optimal sterilization of milk and/or colostrum and prevents stagnation in the UV reactor related piping, connections, and control systems. Preferably, the flow rate is about 17 gallons per minute, but other flow rates may be used as the number, size, and efficiency of UV reactors changes.

Temperature controllers for use in the present invention can include sensors and heat exchangers to warm or cool the milk to an optimum temperature range for cooling and storage, for calves to be fed with milk directly from the pasteurizing apparatus or for accommodating temperature losses in milk lines, containers or other equipment disposed between the apparatus and the calves. The pasteurizing temperature can be adjusted accordingly because in the present invention, milk temperature does not contribute to pasteurizing.

The present invention also can include a stand pipe with milk volume detector for determining the amount of milk in the vat, and calculating and controlling treatment time based on milk quantity. For example, a 100 gallon vat can be filled or partially filled with 100 gallons or 5 gallons of milk, and the present invention will automatically set an approximate treatment time.

Apparatus of the present invention can also include a mobile storage vat for transporting pasteurized milk to the calves at remote locations. The mobile vat can be insulated and include a spray ball or device for cleaning the mobile vat. A mobile platform may simply transport the vat in which the milk was stored during pasteurization.

Other features and benefits of the invention will be apparent from the detailed description and drawings of this disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic view of a milk treatment system in accordance with the present invention;

FIG. 2 is a perspective view of a milk treatment system in accordance with the present invention;

FIG. 3 is a perspective view of the milk treatment system of FIG. 2 with its controller removed;

FIG. 4 is a front view of the milk treatment system of FIG. 2 and including a storage vat for milk in accordance with the present invention;

FIG. 5 is a schematic view of the milk treatment system with a mobile platform for transporting the storage vat, in accordance with the present invention;

FIG. 6 is a front and partial cross-sectional view of a storage vat and stand pipe in accordance with the present invention; and

FIG. 7 is a schematic view of the milk treatment system illustrated in FIG. 1, but with a cross-sectional view of the UV reactor.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the following description of the invention, the same reference numeral will be used to identify the same or similar

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elements in the each of the figures. FIGS. 1 through 5, and 7 illustrate a waste milk treatment system 20 in accordance with the present invention, including: a controller 22; a milk pump 24; an inlet conduit 26; an outlet conduit 28; a pump inlet conduit 29; an ultraviolet milk treatment device 30 (referred to herein as "UV reactor"); and an outlet conduit 32. A storage vat 34 and a mobile platform 36 (FIG. 5) are used in connection with the milk treatment system 20.

Generally, the depicted milk treatment system 20 uses the milk pump 24 to pump milk through the inlet conduit 26, and into the UV reactor 30. While being treated in the UV reactor 30, the milk is heated or cooled in a heat exchanger to be in a temperature range that minimizes the growth of bacteria without destroying the immunoglobulins that are important for calf health. The treated milk moves through the outlet conduit 28 to be temperature adjusted and stored in the storage vat 34. The milk storage vat 34 may be supported on the mobile platform 36 for transporting the milk to calves before the treated milk falls out of a predetermined temperature range. The mobile platform 36 may also include a milk distribution pump 39 to pump milk through a feed tube 41 to calves.

More specifically, the controller 22 activates the pump 24 to circulate milk to the UV reactor 30 which is preferably surrounded by a heat exchanger 38 to raise milk temperature to between 85° F. and 120° F. and then circulates the warmed milk through the UV reactor 30 at an appropriate rate and frequency to kill harmful bacteria. The milk can then be chilled and stored or fed directly to calves. By raising the milk temperature to only the range of 85° F. to 120° F., there is much less destruction of immunoglobulins and the milk is still safely pasteurized by the UV light from the UV reactor 30.

Unlike batch pasteurizers, UV reactors do not rely on the temperature of the milk to kill bacteria. Instead, the UV light alone in the range of 200 to 280 nanometers, UVC range (germicidal range), kills the bacteria. Nonetheless, milk temperature is important because cold milk is churned by the pump and piping system and butter flakes can form that are less likely to be adequately treated by the UV light. Raising milk temperature to 85° F. or higher is sufficient to melt or reduce the size of butter flakes that form so that the milk is adequately treated by the UV reactors. Preferably, the milk temperature is raised to above 95° F., and more preferably to above 100° F. On the other hand, heating the milk to too high of a temperature can destroy beneficial immunoglobulins. An upper end of the temperature range to minimize destruction of the immunoglobulins is 120° F.

A temperature range of about 85° F. to about 120° F. includes a feeding temperature range of between about 100° F. and about 110° F. If the milk is to be fed directly to calves, then heating to the feeding temperature range of between about 100° F. to 110° F. for pasteurizing is appropriate.

If the milk is to be chilled and stored after pasteurization, a milk temperature in the lower end of the range of 85° F. to 120° F. will produce adequate results in the present invention. It is noted that treated waste milk may need to be transported to the calves where they are kept in the dairy facility. In this situation, heating the milk to above the feeding temperature range can compensate for milk cooling as it is being transported. Heat loss is a function of ambient conditions, the time between pasteurization and feeding, and other factors. Thus, using the present invention, milk temperature can be adjusted to compensate for these and other factors in any particular dairy situation.

A preferred heat exchanger 38 for use with the present invention is disposed around the UV reactor 30. The heat exchanger 38 is depicted in FIG. 7 and includes an inlet 62, a

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ratus to accommodate calf needs, temperature losses for time, distance and methods for transporting the milk to calves.

UV milk pasteurizers for use in the present invention can be those of the type disclosed in Rix et al., U.S. Pat. No. 6,916, 452 (incorporated herein by reference). A number of such pasteurizers can be used in series to reduce the number of times milk is circulated through the UV reactors.

Preferably, the controller of present invention monitors UV reactor components and adjusts treatment time to accommodate defective UV bulbs, ballasts, or related components.

Flow controllers for use in the present invention include pumps and meters that pump milk through the UV reactor at a rate that ensures optimal sterilization of milk and/or colostrum and prevents stagnation in the UV reactor related piping, connections, and control systems. Preferably, the flow rate is about 17 gallons per minute, but other flow rates may be used as the number, size, and efficiency of UV reactors changes.

Temperature controllers for use in the present invention can include sensors and heat exchangers to warm or cool the milk to an optimum temperature range for cooling and storage, for calves to be fed with milk directly from the pasteurizing apparatus or for accommodating temperature losses in milk lines, containers or other equipment disposed between the apparatus and the calves. The pasteurizing temperature can be adjusted accordingly because in the present invention, milk temperature does not contribute to pasteurizing.

The present invention also can include a stand pipe with milk volume detector for determining the amount of milk in the vat, and calculating and controlling treatment time based on milk quantity. For example, a 100 gallon vat can be filled or partially filled with 100 gallons or 5 gallons of milk, and the present invention will automatically set an approximate treatment time.

Apparatus of the present invention can also include a mobile storage vat for transporting pasteurized milk to the calves at remote locations. The mobile vat can be insulated and include a spray ball or device for cleaning the mobile vat. A mobile platform may simply transport the vat in which the milk was stored during pasteurization.

Other features and benefits of the invention will be apparent from the detailed description and drawings of this disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic view of a milk treatment system in accordance with the present invention;

FIG. 2 is a perspective view of a milk treatment system in accordance with the present invention;

FIG. 3 is a perspective view of the milk treatment system of FIG. 2 with its controller removed;

FIG. 4 is a front view of the milk treatment system of FIG. 2 and including a storage vat for milk in accordance with the present invention;

FIG. 5 is a schematic view of the milk treatment system with a mobile platform for transporting the storage vat, in accordance with the present invention;

FIG. 6 is a front and partial cross-sectional view of a storage vat and stand pipe in accordance with the present invention; and

FIG. 7 is a schematic view of the milk treatment system illustrated in FIG. 1, but with a cross-sectional view of the UV reactor.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the following description of the invention, the same reference numeral will be used to identify the same or similar

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elements in the each of the figures. FIGS. 1 through 5, and 7 illustrate a waste milk treatment system 20 in accordance with the present invention, including: a controller 22; a milk pump 24; an inlet conduit 26; an outlet conduit 28; a pump inlet conduit 29; an ultraviolet milk treatment device 30 (referred to herein as "UV reactor"); and an outlet conduit 32. A storage vat 34 and a mobile platform 36 (FIG. 5) are used in connection with the milk treatment system 20.

Generally, the depicted milk treatment system 20 uses the milk pump 24 to pump milk through the inlet conduit 26, and into the UV reactor 30. While being treated in the UV reactor 30, the milk is heated or cooled in a heat exchanger to be in a temperature range that minimizes the growth of bacteria without destroying the immunoglobulins that are important for calf health. The treated milk moves through the outlet conduit 28 to be temperature adjusted and stored in the storage vat 34. The milk storage vat 34 may be supported on the mobile platform 36 for transporting the milk to calves before the treated milk falls out of a predetermined temperature range. The mobile platform 36 may also include a milk distribution pump 39 to pump milk through a feed tube 41 to calves.

More specifically, the controller 22 activates the pump 24 to circulate milk to the UV reactor 30 which is preferably surrounded by a heat exchanger 38 to raise milk temperature to between 85° F. and 120° F. and then circulates the warmed milk through the UV reactor 30 at an appropriate rate and frequency to kill harmful bacteria. The milk can then be chilled and stored or fed directly to calves. By raising the milk temperature to only the range of 85° F. to 120° F., there is much less destruction of immunoglobulins and the milk is still safely pasteurized by the UV light from the UV reactor 30.

Unlike batch pasteurizers, UV reactors do not rely on the temperature of the milk to kill bacteria. Instead, the UV light alone in the range of 200 to 280 nanometers, UVC range (germicidal range), kills the bacteria. Nonetheless, milk temperature is important because cold milk is churned by the pump and piping system and butter flakes can form that are less likely to be adequately treated by the UV light. Raising milk temperature to 85° F. or higher is sufficient to melt or reduce the size of butter flakes that form so that the milk is adequately treated by the UV reactors. Preferably, the milk temperature is raised to above 95° F., and more preferably to above 100° F. On the other hand, heating the milk to too high of a temperature can destroy beneficial immunoglobulins. An upper end of the temperature range to minimize destruction of the immunoglobulins is 120° F.

A temperature range of about 85° F. to about 120° F. includes a feeding temperature range of between about 100° F. and about 110° F. If the milk is to be fed directly to calves, then heating to the feeding temperature range of between about 100° F. to 110° F. for pasteurizing is appropriate.

If the milk is to be chilled and stored after pasteurization, a milk temperature in the lower end of the range of 85° F. to 120° F. will produce adequate results in the present invention. It is noted that treated waste milk may need to be transported to the calves where they are kept in the dairy facility. In this situation, heating the milk to above the feeding temperature range can compensate for milk cooling as it is being transported. Heat loss is a function of ambient conditions, the time between pasteurization and feeding, and other factors. Thus, using the present invention, milk temperature can be adjusted to compensate for these and other factors in any particular dairy situation.

A preferred heat exchanger 38 for use with the present invention is disposed around the UV reactor 30. The heat exchanger 38 is depicted in FIG. 7 and includes an inlet 62, a

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water jacket 64, and an outlet 66. The water jacket 64 surrounds and is substantially coaxial with the UV reactor 30 to define an annular space through which water, air or other heat exchanger fluid can flow to adjust the temperature of the UV reactor 30 and the milk being pasteurized therein. Preferably, the water jacket 64 is made of stainless steel, but other materials can be used. The inlet 62 is in communication with a source of water or other fluid, and the fluid source can be a hot water heater, for example. Preferably, the hot water heater is a dedicated unit for the heat exchanger 38, in a closed loop configuration with the heat exchanger 38.

Other types of heat exchangers can be used with the present invention, and be positioned at any point in the milk flow path.

A temperature sensor 44 is used to determine milk temperature throughout the pasteurizing process. Preferably, the temperature sensor 44 is a Precision Fahrenheit Temperature Sensor Model LM34, available from National Semiconductor. If the pasteurization process has been completed and the desired milk temperature has not been reached, milk will continue to be circulated until the desired temperature is reached.

Further, the controller 22 is preferably in communication with a milk quantity sensor 43, which is preferably an integrated silicon pressure sensor (MPX5010GP, Case 867b-04) available from Freescale Semiconductor (www.freescale.com) that uses a long stand tube 42 that traps air when the liquid level of the vat 34 increases. As the milk level in the vat 34 rises, pressure in the long stand tube 42 increases. The milk quantity sensor 43 generates a voltage readout signal that is communicated to the controller 22 to automatically determine run time for any batch size of milk.

Preferably, the controller 22 is set initially by a skilled installer or technician. Adjustments can be made by any dairy operator at an operator interface 45 to adjust temperature, flow rates, treatment time or any other condition in the pasteurizing process.

The controller 22 also provides a display 42 indicating how long it has been since the pasteurizing process has been completed and the milk's current temperature so that re-circulation through the heat exchanger 38 can bring milk temperature back within a desired range. The display 42 can provide a dairy operator with any relevant information, including operating times, flow rates, milk temperature, component failure, maintenance requirements, and so on.

Finally, after pasteurized milk has been distributed to calves or the mobile platform 36, the milk treatment system 20 can be coupled to a wash system (not illustrated) for automatic cleaning and preparation for the next pasteurizing cycle.

The milk treatment system 20 can include one or more UV milk pasteurizer reactors 30. Three UV reactors 30 are used in the illustrated embodiment. UV milk pasteurizers for use in the present invention can be those of the type disclosed in Rix et al., U.S. Pat. No. 6,916,452 (incorporated herein by reference). The UV reactors 30 as depicted in FIG. 7 include an inlet 50, an outlet 60, a quartz tube 52, a UV light bulb 56 disposed inside the quartz tube 52 to prevent milk from contacting the light bulb 56. Other types of tubes can be used to protect the light bulb 56 from being damaged by milk. Surrounding the quartz tube 52 is an outer tube 58 (preferably made of stainless steel) that defines with the quartz tube 52, an annular milk flow channel. The heat exchanger 38 surrounds the outer tube 58. Milk flows through the inlet 50, the annular flow channel 58 where it is pasteurized by UV light, and out the outlet 60.

The UV light bulb 56 is preferably a GIA972T5LCA/2S07/PT-18"4W/N/CB-061 (UV Pure) available from First

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Light Technologies, Inc., P.O. Box 191, 212 Ideal Way, Poultney, Vt. 05764. Ballasts for use in the UV reactor 30 preferably are Electronic Ballasts, EVG 100 . . . 200W/230V AC, available from ZED—Ziegler Electronic Devices GmbH.

The quartz tube 52 is about one inch in outside diameter and the inside diameter of the outer tube 58 is about 1.37 inches. Further, the outside diameter of the outer tube 58 is about 1.50 inches in diameter and the inside diameter of the water jacket 64 is about 2.37 inches, but other dimensions of the water jacket 64 are possible.

Other combinations of bulbs and ballasts are possible, and it is desirable that the combination be UL rated. The UV reactors 30 can be used in series or parallel to reduce the number of times milk is circulated through the UV reactors. Preferably, the controller 22 of the present invention is in monitoring communication to monitor UV reactor 30 components and adjust treatment time to accommodate defective UV light bulbs 56, ballasts or related components. One way to monitor such components is to monitor electrical current flow through a light bulb, for example. If the bulb is not working no current will be flowing through the bulb.

The pump 24 pumps milk through the UV reactor 30 at a rate that ensures optimal sterilization of milk and/or colostrum and prevents stagnation in the UV reactor 30 related piping, connections, and control systems. Preferably, the flow rate is about 17 gallons per minute, but other flow rates may be used as other system components are changed in size or type.

Preferably, the controller 22 is programmed to operate the milk pump 24 at about seventeen gallons per minute flow rate. Using one UV reactor 30 alone, this flow rate will result in pasteurized milk after about 40 "passes" through the UV reactor 30. Using two UV reactors 30 in series will require about 20 passes, and using three UV reactors in series will require about 13.4 passes through the reactors 30.

For fifty gallons of waste milk at 2.9 minutes per pass, the UV pasteurizing process will take about 59 minutes. This is an improvement over batch pasteurizing heating, treatment, and cooling times. Further, the present invention saves time and energy primarily because the milk does not require heating to such high temperatures. Tests have shown 30% to 70% time savings for the present invention over the batch pasteurizing process.

Further, the present invention promotes efficiencies in dairies because a milk vat 34 can be filled hours before milk is needed, and the pasteurizing process can be initiated automatically by the controller 22 at an appropriate time to warm, pump, pasteurize and store the milk on a mobile platform 36 for transport to calves. This function is preferably set by a pasteurization start timer accessible at the operator interface 45.

As depicted in FIG. 6, the present invention also can include a stand pipe 42 with milk volume detector 38 for determining the amount of milk in the vat 34. The controller 22 calculates and controls treatment time based on the milk quantity in the vat 34. For example, a 100 gallon vat 34 can be filled or partially filled with 100 gallons or 5 gallons of milk, and the present invention will automatically set the approximate treatment time.

Apparatus of the present invention can also include a mobile platform 36 (FIG. 5) or storage vat 34 for transporting the milk from the pasteurizer to the calves at remote locations. The mobile vat 36 can be insulated and include a spray ball or device for cleaning the mobile vat 36. A mobile platform 36 may simply be a frame and wheels to transport the vat 34 in which the milk was stored during pasteurization.

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Comparisons between the present invention and the prior art batch pasteurizers illustrate the efficacy of the present invention for use with waste milk and colostrum.

EXAMPLE A

In this example A, the standard batch pasteurizer is more effective than the UV pasteurizer for all the organisms tested in killing harmful bacteria, but when milk is used, the invention is effective for all three organisms tested (99.98% for *E. coli*, 100% for *B. cereus* and 99.992% for *Salmonella*). However, when colostrum is used the invention is certainly less effective for all three to the point where it may not be effective enough. More research may be needed to determine the maximum effectiveness, in terms of increasing treatment time, for effective bactericidal action on microorganisms when present in colostrum.

Further, single Radial immunodiffusion assays were also run for these samples for bovine IgG. The batch pasteurizer samples displayed a significant reduction in IgG (around 43%) whereas the UV samples had no reduction in IgG at all. Thus, the present invention as shown in Example A results in healthier waste milk, but possibly not healthier colostrum.

	CFU/ml	% Survival	% Kill
Batch Pasteurizer time-substrate <i>E. coli</i> ATCC # 25922			
Pre heat-milk ^a	3.93 × 10 ⁶	—	—
Time 0 minutes-milk ^b	4.60 × 10 ³	0.007%	99.88%
Time 10 minutes-milk	0	0%	100%
Time 20 minutes-milk	0	0%	100%
Time 30 minutes-milk	0	0%	100%
Pre-heat-colostrum ^a	2.31 × 10 ⁶	—	—
Time 0 minutes-colostrum ^b	0	0%	100%
Time 10 minutes-colostrum	0	0%	100%
Time 20 minutes-colostrum	0	0%	100%
Time 30 minutes-colostrum	0	0%	100%
UV Pasteurizer time-substrate <i>E. coli</i> ATCC # 25922			
Time 0 minutes-milk	4.41 × 10 ⁶	—	—
Time 5 minutes-milk	1.30 × 10 ⁵	2.95%	97.1%
Time 10 minutes-milk	3.73 × 10 ³	0.085%	99.92%
Time 15 minutes-milk	9.30 × 10 ²	0.021%	99.98%
Time 0 minutes-colostrum	8.68 × 10 ⁶	—	—
Time 5 minutes-colostrum	6.65 × 10 ⁶	76.6%	23.4%
Time 10 minutes-colostrum	2.60 × 10 ⁶	30.0%	70.0%
Time 15 minutes-colostrum	1.88 × 10 ⁶	21.7%	78.3%
Batch Pasteurizer time-substrate <i>Bacillus cereus</i> ATCC # 4342			
Pre heat-milk ^a	1.08 × 10 ⁵	—	—
Time 0 minutes-milk ^b	17.5	0%	100%
Time 10 minutes-milk	0	0%	100%
Time 20 minutes-milk	0.5	0%	100%
Time 30 minutes-milk ^a	22.5	0%	100%
Pre-heat-colostrum ^a	1.10 × 10 ⁵	—	—
Time 0 minutes-colostrum ^b	12.5	0%	100%
Time 10 minutes-colostrum	2.5	0%	100%
Time 20 minutes-colostrum	2.5	0%	100%
Time 30 minutes-colostrum	2.5	0%	100%
UV Pasteurizer time-substrate <i>Bacillus cereus</i> ATCC # 4342			
Time 0 minutes-milk	1.61 × 10 ⁵	—	—
Time 5 minutes-milk	1.11 × 10 ⁴	6.89%	93.1%
Time 10 minutes-milk	1.80 × 10 ²	0.1118%	99.89%
Time 15 minutes-milk	12.5	0%	100%
Time 0 minutes-colostrum	1.88 × 10 ⁵	—	—

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-continued

	CFU/ml	% Survival	% Kill
5	Time 5 minutes-colostrum	5.83 × 10 ⁴	31.0%
	Time 10 minutes-colostrum	2.48 × 10 ⁴	13.2%
	Time 15 minutes-colostrum	1.47 × 10 ⁴	7.8%
	Batch Pasteurizer time-substrate <i>Salmonella choleraesuis</i> ATCC # 6539		
10	Pre heat-milk ^a	3.08 × 10 ⁶	—
	Time 0 minutes-milk ^b	1.50 × 10 ²	0.0049%
	Time 10 minutes-milk	5	0%
	Time 20 minutes-milk	0	0%
15	Time 30 minutes-milk	0	0%
	Pre-heat-colostrum ^a	7.27 × 10 ⁵	—
	Time 0 minutes-colostrum ^b	0	0%
	Time 10 minutes-colostrum	0	0%
20	Time 20 minutes-colostrum	0	0%
	Time 30 minutes-colostrum	0	0%
	UV Pasteurizer time-substrate <i>Salmonella choleraesuis</i> ATCC # 6539		
	Time 0 minutes-milk	3.22 × 10 ⁶	—
25	Time 5 minutes-milk	4.52 × 10 ⁴	1.40%
	Time 10 minutes-milk	8.25 × 10 ²	0.026%
	Time 15 minutes-milk	2.68 × 10 ²	0.0083%
	Time 0 minutes-colostrum	2.88 × 10 ⁶	—
30	Time 5 minutes-colostrum	6.68 × 10 ⁵	23.2%
	Time 10 minutes-colostrum	1.39 × 10 ⁵	4.83%
	Time 15 minutes-colostrum	8.41 × 10 ⁴	2.92%

^aSubstrate (milk or colostrum) before any heating.

^bSubstrate (milk or colostrum) right after getting up to the pasteurization temperature (time = 0). This takes about one-half an hour.

EXAMPLE B

For this example, the UV Pasteurizer trial for the milk was repeated, with the addition of another time at 22 minutes exposure of the milk to UV light. These results are very similar to Example A with a satisfactory level of kill after 15 minutes exposure for 10 gallons of milk with all three bacteria tested.

The second part of the study utilized colostrum which Example A had an ineffective level of kill after 15 minutes for the organisms tested. Example B utilized exposure levels of 30 and 45 minutes. These results indicated better efficacy in two of the three organisms at 15 minutes versus Example A. Example A shows the efficacy at 15 minutes to be marginal for two organisms and unacceptable for the third (*Bacillus*). At both 30 and 45 minutes exposure, however, the efficacy of the pasteurizer of the present invention for all three organisms was adequate to very good.

Further, Single Radial immunodiffusion assays were also run for these samples for bovine IgG. The results indicated no reduction in immunoglobulin (IgG) after 15 minutes and a relatively minimal reduction in immunoglobulin (IgG) after 30 and 45 minutes. Thus, the present invention as reflected in Example B results in healthier milk and colostrum than the prior art batch pasteurizer.

	CFU/ml	% Survival	% Kill
UV Pasteurizer time-substrate <i>E. coli</i> ATCC # 25922			
60	Time 0 minutes-milk	9.55 × 10 ⁶	—
	Time 5 minutes-milk	1.81 × 10 ⁵	1.90%
	Time 10 minutes-milk	1.34 × 10 ³	0.014%
	Time 15 minutes-milk	4.53 × 10 ²	0.005%

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-continued

	CFU/ml	% Survival	% Kill
Time 22 minutes-milk	6.50×10^4	0.0007%	99.9993%
Time 0 minutes-colostrum	1.47×10^7	—	—
Time 15 minutes-colostrum	1.28×10^5	0.87%	99.1%
Time 30 minutes-colostrum	7.03×10^3	0.048%	99.95%
Time 45 minutes-colostrum	2.92×10^2	0.002%	99.998%
UV Pasteurizer time-substrate			
<i>Bacillus cereus</i> ATCC # 4342			
Time 0 minutes-milk	5.07×10^3	—	—
Time 5 minutes-milk	2.26×10^3	44.58%	55.4%
Time 10 minutes-milk	2.80×10^1	0.56%	99.4%
Time 15 minutes-milk	3.3	0.065%	99.93%
Time 22 minutes-milk	0	0%	100%
Time 0 minutes-colostrum	1.43×10^4	—	—
Time 15 minutes-colostrum	5.18×10^3	36.0%	63.8%
Time 30 minutes-colostrum	3.67×10^2	2.57%	97.4%
Time 45 minutes-colostrum	8.3	0.058%	99.94%
UV Pasteurizer time-substrate			
<i>Salmonella choleraesuis</i>			
ATCC # 6539			
Time 0 minutes-milk	4.66×10^3	—	—
Time 5 minutes-milk	6.30×10^1	1.35%	98.6%
Time 10 minutes-milk	0	0%	100%
Time 15 minutes-milk	2.83×10^1	0.61%	99.3%
Time 22 minutes-milk	6.7	0.14%	99.86%
Time 0 minutes-colostrum	3.72×10^6	—	—
Time 15 minutes-colostrum	2.68×10^4	0.72%	99.28%
Time 30 minutes-colostrum	5.73×10^2	0.0154%	99.98%
Time 45 minutes-colostrum	3.0×10^1	0.0008%	99.9992%

EXAMPLE C

This example utilized colostrum at the same exposure levels of 15, 30 and 45 minutes as Example B. These results indicated that the UV Pasteurizer of the present invention overall is still effective at 30 and 45 minutes, especially the latter time. The only possible exception is possibly the *Bacillus* where only the 45 minute exposure time showed solid results. The results generally showed less percent kill than the previous test at the same exposure times, but the Time 0 concentrations were also higher, which probably accounts for this reduction in kill. In real field use, the concentration of these pathogens is very unlikely to be anywhere near what is being tested in this example. It can therefore be concluded that the kill rates are satisfactory at both 30 and 45 minutes.

Further, Single Radial immunodiffusion assays were also run for these samples for bovine IgG. The results indicated minimal or no reduction in immunoglobulin (IgG) after 15, 30 and 45 minutes. This is therefore, a distinct improvement over batch pasteurizing.

	CFU/ml	% Survival	% Kill
UV Pasteurizer time-substrate			
<i>E. coli</i> ATCC # 25922			
Time 0 minutes-colostrum	3.40×10^7	—	—
Time 15 minutes-colostrum	3.26×10^6	9.59%	90.4%
Time 30 minutes-colostrum	1.26×10^5	0.37%	99.6%
Time 45 minutes-colostrum	3.68×10^3	0.011%	99.989%
UV Pasteurizer time-substrate			
<i>Bacillus cereus</i> ATCC # 4342			
Time 0 minutes-colostrum	2.16×10^4	—	—
Time 15 minutes-colostrum	1.22×10^4	56.5%	43.5%
Time 30 minutes-colostrum	1.14×10^3	5.28%	94.7%
Time 45 minutes-colostrum	8.50×10^1	0.39%	99.6%

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-continued

	CFU/ml	% Survival	% Kill
UV Pasteurizer time-substrate			
<i>Salmonella choleraesuis</i>			
ATCC # 6539			
Time 0 minutes-colostrum	1.16×10^7	—	—
Time 15 minutes-colostrum	6.33×10^5	5.46%	94.5%
Time 30 minutes-colostrum	1.56×10^4	0.13%	99.87%
Time 45 minutes-colostrum	6.75×10^2	0.0058%	99.994%

^cColony-forming units per ml.

The above examples illustrate the effectiveness of the present invention in killing three types of pathogens while preserving essentially all of the immunoglobulins in milk and colostrum.

The previous detailed description of the preferred embodiments of the invention are presented for clearness of understanding, and are not intended to limit the scope of the following claims. Further, the term "milk" as used in the claims is intended to be broad enough to include milk, waste milk, non-saleable milk, colostrum or any other calf feed supplement that would benefit from pasteurizing prior to feeding to calves.

The invention claimed is:

1. A milk treatment system comprising:

an ultraviolet milk treatment device having an inlet and an outlet;

a storage vat in fluid communication with the ultraviolet milk pasteurizing treatment device;

a pump in fluid communication with the storage vat;

a milk heat exchanger in which the milk treatment device is at least partially disposed;

and

a controller in communication with the pump and heat exchanger.

2. The milk treatment system of claim 1, wherein the ultraviolet milk treatment device comprises:

a plurality of ultraviolet milk treatment reactors.

3. The milk treatment system of claim 1, and further comprising:

a mobile platform supporting the storage vat.

4. The milk treatment system of claim 1, and further comprising:

a mobile platform supporting the milk vat; and

the controller comprises: a milk temperature adjuster.

5. The milk treatment system of claim 1, wherein the controller is in monitoring communication with the ultraviolet milk treatment device.

6. The milk treatment system of claim 1, and further comprising:

a milk quantity sensor in fluid communication with the storage vat and the controller.

7. The milk treatment system of claim 1, wherein the controller includes an operator interface for adjusting the controller.

8. The milk treatment system of claim 1, wherein the controller comprises:

a pasteurization start timer.

9. A milk treatment system comprising:

an ultraviolet milk treatment device having an inlet and an outlet;

a storage vat in fluid communication with the ultraviolet milk treatment device;

a pump in fluid communication with the storage vat;

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a milk heat exchanger, wherein the milk heat exchanger comprises a water jacket surrounding at least a portion of the ultraviolet milk treatment device; and
a controller in communication with the pump and heat exchanger.
10. A milk treatment system comprising:
an ultraviolet milk treatment device having an inlet and an outlet;
a storage vat in fluid communication with the ultraviolet milk treatment device;
a pump in fluid communication with the storage vat;
a milk heat exchanger; and
a controller in communication with the pump and heat exchanger, wherein the controller adjusts the milk heat exchanger to a milk temperature range of about 85° F. and not to exceed about 120° F.

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11. A milk treatment system comprising:
an ultraviolet milk treatment device having an inlet and an outlet;
a storage vat in fluid communication with the ultraviolet milk treatment device;
a pump in fluid communication with the storage vat;
a milk heat exchanger; and
a controller in communication with the pump and the milk heat exchanger to maintain milk in the ultraviolet milk treatment device for a milk exposure time of between about 15 minutes and about 60 minutes.
12. The milk treatment system of claim 11, wherein the milk exposure time is between about 30 minutes and about 45 minutes.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 8,127,667 B2
APPLICATION NO. : 12/383830
DATED : March 6, 2012
INVENTOR(S) : Kevin M. Kastenschmidt et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Col. 10, in Claim 1, line 32, the word "pasteurizing" should be deleted.

Annexure C

The ‘conventional’ calculations measuring UV-C dose per area and other important critical control parameters relating to the SurePure UV System.

SUREPURE TURBULATOR - DOSE MEASUREMENT AREA (CONVENTIONAL)			
C1	<u>Power supply specifications:</u>		
	Voltage (VL) @ 120 VAC/60Hz	230	V
	Base to base measurement*	843	mm
	Arc length	767	mm
	Lamp Ø	15	mm
	Current (IL)	400	mA
	Power to lamp	100	W
	UV Output UV-C Watts	25,5	UV-C Watts
	UV Output @ 1 metre**	300	µW.cm ⁻²
	Rated Life hours	9000	hours
	* Measurement from base to base excluding pins		
	** UV output is measured at 253.7 nm at 100 hours under laboratory conditions		
C2	<u>Area in contact with UVC exposure:</u>		
	$A_{uv} = 2 \pi r.h + 2\pi.r^2$		
	where		
	Radius of quartz sleeve in which lamp is housed (r)	1.225	cm
	Length of turbulator + quartz sleeve (h)	86	cm
	therefore		
	Area effective (A_{uv}) (cm ²) OR	671.36	cm ²
	Area effective (A_{uv}) (m ²)	0.067	m ²
C3.0	<u>Intensity:</u>		
	$I = \text{UV-C output} / A_{uv} \text{ (cm}^2\text{)}$		
	where		
	Total UV-C output per unit (W)	25.500	UV-C Watts
	Area (cm ²)	671.362	cm ²
	therefore		
	Intensity (W.cm ⁻²) OR	0.038	W.cm ⁻²
	Intensity (mW.cm ⁻²)	37.98	mW.cm ⁻²
C4	<u>Flow rate Unit:</u>		
	Actual flow rate (litres per hour)	4000	L.h ⁻¹
	Actual flow rate (litres per second)	1.111	L.s ⁻¹
	Flow rate (Q)	0.00111	m ³ .s ⁻¹
C5	<u>Volume of turbulator:</u>		
	$V = \pi r^2 h$		
	where		
	Length / Height of cylinder (h)	0.860	m

	<i>therefore</i>		
	Volume (physical measurement) (litres)	0.675	L
	Volume (physical measurement) (m ³)	6.75E-04	m ³
C6	<u>Retention time (t)</u>		
	Retention Time = Volume reactor / Flow rate (l.s ⁻¹)	0.608	s
C7	<u>Effective UV-C dosage (area):</u>		
	$D_a = I \times t$		
	<i>where</i>		
	I = effective avg. intensity (μW.cm ²)	37.98	mW.cm ⁻²
	t = Retention Time (s)	0.608	s
	<i>therefore</i>		
	Effective UV-C dosage volume (D_a) (mW-sec.cm ²)	23.07	mW-s.cm ⁻²
C8	<u>Effective Area in unit:</u>		
	$A_e = \frac{1}{4} \pi [(d_2)^2 - (d_1)^2]$		
	<i>where</i>		
	Diameter 1: d ₁	24.5	mm
	Diameter 2: d ₂	40	mm
	<i>therefore</i>		
	Area effective (A_e)	7.85E+02	mm ²
	Area effective (A_e)	7.85E-04	m ²
C9	<u>Velocity of flow:</u>		
	Velocity (V_e) = F_r / A_e		
	<i>where</i>		
	Flowrate (Q)	1.11E-03	m ³ .s ⁻¹
	Area effective (A_e)	7.852E-04	m ²
	<i>therefore</i>		
	Velocity (V_e)	1.415	m.s ⁻¹
C10	<u>Reynolds value</u>		
	$R_e = \rho \cdot V_e \cdot D / \mu$		
	<i>where</i>		
	Density (ρ) milk @ 20°C	1.030	kg.L ⁻¹
	Density (ρ) milk @ 20°C	1030	kg.m ⁻³
	Velocity (V_e)	1.415	m.s ⁻¹
	Characteristic length (D) = d ₂ - d ₁	0.016	m
	Dynamic Viscosity (μ)	3.2	cP
	Dynamic Viscosity (μ)	0.0032	Pa-s
	<i>therefore</i>		
	Reynolds Value minimum estimate (R_e)	7060	

Annexure D

The calculations reported by Reinemann *et al.* (2006) measuring UV-C dose as per the SurePure UV System. Other important critical control parameters relating to the SurePure UV systems remains the same as per the values indicated in Appendix C.

SUREPURE TURBULATOR - DOSE MEASUREMENT VOLUME (Reinemann <i>et al.</i>, 1996)		
R1 <u>Power supply specifications:</u>		
Voltage (VL) @ 120 VAC/60Hz	230	V
Base to base measurement*	843	mm
Arc length	767	mm
Lamp Ø	15	mm
Current (IL)	400	mA
Power to lamp	100	W
UV Output UV-C Watts	25,5	UV-C Watts
UV Output @ 1 metre**	300	µW.cm ²
Rated Life hours	9000	hours
* Measurement from base to base excluding pins		
** UV output is measured at 253.7 nm at 100 hours under laboratory conditions		
R3.1 <u>Effective UV-C Output:</u>		
P_{uv} = UV-C Output X (100% - % losses estimated)		
<i>where</i>		
Losses from light production to light absorption (estimate)	7.50%	
<i>therefore</i>		
Total UV-C energy transmission rate (P_{uv})	23.59	Watts
Total UV-C energy transmission rate (P_{uv})	23.59	J.s ⁻¹
R4 <u>Flow rate Unit:</u>		
Actual flow rate (litres per hour)	4000	L.h ⁻¹
Actual flow rate (litres per second) (Q)	1.111	L.s ⁻¹
Flow rate (Q)	1.11111E-03	m ³ .s ⁻¹
R7 <u>Effective UV-C dosage (per volume):</u>		
$D_v = P_{uv} / Q$		
<i>where</i>		
Total UV-C energy transmission rate (P_{uv})	23.59	J.s ⁻¹
Actual flow rate (litres per second) (Q)	1.111	L.s ⁻¹
<i>therefore</i>		
Effective UV-C dosage volume (D_v)	23	J.L ⁻¹

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